



OPEN *Caenorhabditis elegans* microbiome members have combinatorial effects on host survival and fitness

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Microbiomes are a fundamental part of eukaryotic life and play a role in both host health and fitness. Although microbes are often associated with health and positive effects on the host, certain microbiome compositions are associated with disease. Some disease-associated microbiome compositions are correlated with a change in abundance of a member that is part of the healthy microbiome. We used *Caenorhabditis elegans* and its experimental microbiome, CeMbio, to explore the interactions that individual microbiome members have with the host, and how the entire microbiome community interacts with the host. We compared the effects of individual microbiome members on host survival to those of the standard *C. elegans* laboratory diet of *E. coli* OP50 as well as to the experimental microbiome. We found that while all microbiome members and the whole experimental microbiome are detrimental to *C. elegans* survival when compared to *E. coli*, the survival effects of the individual members show more variation when compared to the experimental microbiome. We also measured effects on host fitness by measuring fecundity and development time across the same comparisons. We found consistent effects on fecundity, but development time was more variable when compared to *E. coli*, but consistently slower when compared to the experimental microbiome. We found that comparisons of the individual microbiome members' effects on host survival and fitness to the effects of the experimental microbiome suggests that the members act in combination with one another. These combinatorial interactions result in specific effects of the microbiome that are different from those of the individual microbiome members that in some cases may be complementary. This further suggests there are potentially different mechanisms resulting in the observed differences in how host survival and fitness respond to individual microbiome members, as well as the whole microbiome. Elucidation of the mechanisms involved in these combinatorial microbe-microbe and host-microbe interactions will lead to greater understanding of the nature of the host-microbiome and host-microbe relationships.

Keywords CeMbio, Microbiome, Host-microbiome interactions, Host-microbe interactions, *C. elegans*, Experimental microbiome

Abbreviations

NGM	Nematode growth media
LB	Luria–Bertani
OD	Optical density
GLHT	General linear hypothesis test
BH	Benjamini–Hochberg
LMM	Linear mixed effects model
E	<i>E. coli</i> OP50
C	CeMbio
TSA	Tryptic soy agar

Microbiome research has been key to understanding organismal health, often uncovering an association between the microbiome and disease. For instance, it has been well documented that gut disorders in humans, such as inflammatory bowel disease and irritable bowel syndrome, are correlated with dysbiotic gut microbiotas, meaning an imbalance of members in the microbial community^{1–10}. Gut microbiotas are currently the most heavily investigated but work on microbiotas associated with other parts of the body have also been linked

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to human disease^{11–13}. However, many of these studies have been correlative, as determining causality can be difficult in human microbiome studies, especially in terms of disease^{14,15}. The relative complexity of human microbiomes presents another challenge to uncovering the roles of individual species within the microbiome. Most often, taxonomic identifications of bacteria that correlate microbiomes to diseases and other phenotypes is at the family level. However, there are some cases where additional information leads to the investigation of a specific species known to be associated with disease. It is possible that some species within a microbiome, known as pathobionts, could be detrimental and understanding the effects of such microbes on the host and on microbiome composition could yield new insights. Due to the challenges of human microbiome research, notably genetic tractability and complexity, model organisms and their defined microbial communities can be of assistance. Thus, leveraging models such as *Caenorhabditis elegans* for in depth studies is an attractive alternative.

In nature, *C. elegans* are found in decaying environments such as compost, rotting fruits, and rotting plant stems¹⁶. Bacterial diversity within these rotting substrates is high¹⁷. Since *C. elegans* is a bacterivorous nematode, its microbiome originates from the bacteria consumed as food. Living in an environment with high bacterial diversity results in the worms having a microbiome that is also microbially diverse^{17,18}. Yet the *C. elegans* microbiome is composed of a set of common genera regardless of where *C. elegans* is isolated¹⁹. Additionally, the microbiomes of *C. elegans* isolated from different locations are more similar to each other than their respective environments, indicating that there is a mechanism(s) that *C. elegans* uses to grow and maintain their gut microbiomes. Based on these commonalities, a defined experimental microbiome was generated. The experimental microbiome, CeMbio, is composed of twelve bacterial strains that represent the natural *C. elegans* microbiome²⁰. The individual members were chosen based on their association with *C. elegans*, ease of growth in a lab setting, and ability to colonize worms' intestines²⁰. Using a small experimental microbiome such as CeMbio allows us to better understand the effects of individual microbiome members, independently as well as in a community, on a host. Prior work has found that *C. elegans* genotype can influence how the microbiome assembles and the general composition of the community^{21,22}, allowing investigations of the role of host genotype in host-microbe interactions. It has recently been shown that individual CeMbio members activate specific host innate immune pathways that appear to act as a protective measure against bacteria that have the potential to shorten the host's lifespan²³. With this knowledge and the CeMbio tool, *C. elegans* is an ideal model to study host-microbe interactions related to microbiomes.

In this study, we sought to determine how individual microbiome members influence host survival and fitness, as well as how the whole microbiome influences host survival and fitness. We measured *C. elegans* survivorship, fecundity, and development time when challenged with individual CeMbio members, as well as the CeMbio community. By making comparisons between effects of individuals and the effects of the microbiome community, we discovered evidence of individual microbiome members acting in a non-additive manner leading to a combination of effects within the community. By making comparisons of natural *C. elegans* strains to the lab-adapted N2 strain, we also discovered that host genotype can also influence the effects that microbes have on *C. elegans* survival and fitness. Collectively, we have started to disentangle how the members of a microbiome work together to affect host health and fitness.

Methods

C. elegans and bacteria strain availability and maintenance

The *C. elegans* N2 strain was received from the Caenorhabditis Genetics Center (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Natural *C. elegans* isolates, MY316, MY2768, and MY2769 (isolated from compost) were provided by Hinrich Schulenberg¹⁷. *E. coli* OP50 was also acquired from the CGC. The twelve CeMbio strains (*Sphingobacterium multivorum* BIGb0170, *Comamonas piscis* BIGb0172, *Pantoea nemavictus* BIGb0393, *Enterobacter hormaechei* CEent1, *Stenotrophomonas indicatrix* JUb19, *Chryseobacterium scophthalmum* JUb44, *Lelliottia amnigena* JUb66, *Sphingomonas molluscorum* JUb134, *Pseudomonas berkeleyensis* MSPm1, *Acinetobacter guillouiae* MYb10, *Pseudomonas lurida* MYb11, *Ochrobactrum vermis* MYb71) were also provided by Hinrich Schulenberg¹⁷ and can also be acquired from the CGC.

Nematode populations were maintained on nematode growth media (NGM) plates seeded with *E. coli* OP50. *E. coli* OP50 isolation-streaked plates were grown on Luria-Bertani (LB) media overnight 37 °C and the CeMbio strains were grown on LB overnight at 25 °C. JUb134 takes approximately 48 h to reach stationary phase. Liquid cultures were made fresh for every experiment. Five milliliters of liquid LB were used to make the cultures and were grown overnight at 37 °C (*E. coli* OP50) or 25 °C (CeMbio strains) for experiments.

The protocol described by Zhang et al.²⁴ was used to make the CeMbio community with the following adjustments. The initial cultures were made in 10mL of liquid LB. Liquid cultures were centrifuged for 30 min at 4000 rpm and the supernatant was then removed. The bacterial pellets were washed twice with 5mL 1X PBS and centrifuged again for 30 min at 4000 rpm after each wash and was resuspended in 5mL 1X PBS. The optical density at 600 nm (OD₆₀₀) was measured and cultures were diluted with 1X PBS to an OD₆₀₀ of 1. The community was assembled by pooling equal volumes of all individual cultures in a new conical tube.

Nematode survivorship

Seventy-five µL of each bacterial culture was seeded on NGM plates two days before use. The same day the bacteria were seeded, nematodes were synchronized by bleaching and maintained on *E. coli* OP50 plates. Two days after synchronizing the populations, twelve L4 nematodes were moved to the seeded NGM plates. All nematode: treatment combinations were performed on triplicate plates, and the experiments were performed twice. Experiment plates were maintained at 25 °C. Nematodes were scored as living or dead every day until all individuals were deceased. Nematodes that died by desiccation on the plate plastic or if a carcass was not found were removed from the experiment. To avoid progeny confounding the results, nematodes were moved to new experiment plates every day until no more eggs were laid.

Relative hazard (β) of a treatment, found from the GLHT was used to quantify these comparisons. A treatment with an increased hazard (more detrimental effects) had an estimate greater than zero, whereas one with decreased hazard had an estimate less than zero. We used the $-\beta$ value to make the comparisons more intuitive, with a negative value indicating a negative effect on survivorship and a positive value indicating a positive effect.

Nematode fecundity

Bacterial cultures were seeded on NGM plates two days prior to use in 35 μ L volumes and spread thin across the plate using a glass pipette to ensure bacterial lawns would be thin enough to count individual worms later. Two days after seeding the bacteria, nematodes were synchronized and maintained on the treatment, either *E. coli* OP50, an individual CeMbio member, or CeMbio. Individual L4 worms were moved to their own treatment plate and were moved to new plates every day for six days. Progeny were counted three days later. The experiment was replicated twice and performed at 20 °C and the nematodes in this experiment were only used to measure fecundity.

Similar statistical analyses were performed using a linear mixed effects model (LMM) from the lme4 v_1.1-35.2 package in R [<http://www.jstatsoft.org/v67/i01/>]³¹. The LMM allowed us to determine if there were similar differences as we did with survivorship. The number of individuals for each strain: bacteria combination shown in Fig. 2 and Supplementary Fig. S2 are as follows: N2:OP50, $n = 60$, N2:CeMbio, $n = 30$, N2:individual CeMbio members, $n = 10$, MY316:OP50, $n = 60$, MY316:CeMbio, $n = 20$, MY316:individual CeMbio members, $n = 10$, MY2768:OP50, $n = 60$, MY2768:CeMbio, $n = 15$, MY2768:individual CeMbio members, $n = 10$, MY2769:OP50, $n = 60$, MY2769:CeMbio, $n = 15$, MY2769:individual CeMbio members, $n = 10$. After making the LMM, the GLHT with BH adjustment was used again to make specific comparisons.

Data was visualized by calculating the z-score for each comparison. The “population” mean and standard deviation used was that of the treatment used as a comparison. For example, when N2 exposed to an individual CeMbio member was compared to N2 exposed to *E. coli* OP50, the mean and standard deviation used were from N2 exposed to *E. coli* OP50. Similarly, when the comparison was to CeMbio, the specific worm strain exposed to CeMbio was used for the mean and standard deviation.

Nematode development time

Two days prior to use, 35 μ L of bacterial cultures were seeded on NGM plates and spread thin across the plate using a glass pipette. Nematodes are synchronized via bleaching and are rotated in M9 for two days at 20 °C to synchronize the population at the L1 stage. Synchronized L1s are seeded on experimental plates 44 h prior to development scoring using a Pasteur pipette. This resulted in approximately 200 worms deposited per nematode-treatment combination. The worms were scored as L4s or adults 44 h post-plating. Worms were scored every two hours until all worms were adults. The experiment was replicated twice and performed at 20 °C and the nematodes used in this experiment were only used to measure development time.

A LMM approach was also taken to analyze development time data. The time-to-adult on the various bacterial treatments was the dependent variable in the model. The number of individuals for each strain: bacteria combination shown in Fig. 3 and Supplementary Fig. S3 are as follows: N2:OP50, $n = 1411$, N2:CeMbio, $n = 1485$, N2:individual CeMbio members, $n = 91-309$, MY316:OP50, $n = 1588$, MY316:CeMbio, $n = 1174$, MY316:individual CeMbio members, $n = 244-479$, MY2768:OP50, $n = 278$, MY2768:CeMbio, $n = 500$, MY2768:individual CeMbio members, $n = 235-408$, MY2769:OP50, $n = 433$, MY2769:CeMbio, $n = 734$, MY2769:individual CeMbio members, $n = 433-643$. That model was then used for a GLHT with BH adjustment to make specific comparisons between treatments and CeMbio or *E. coli* OP50, and between nematode genotypes.

Data was visualized by calculating the z-score for each comparison. The “population” mean and standard deviation used was that of the treatment used as a comparison. For example, when N2 exposed to an individual CeMbio member was compared to N2 exposed to *E. coli* OP50, the mean and standard deviation used were from N2 exposed to *E. coli* OP50. Similarly, when the comparison was to CeMbio, the specific worm strain exposed to CeMbio was used for the mean and standard deviation. We used a negative z-score to make the comparisons more intuitive, with a negative value indicating a negative effect on development time and a positive value indicating a positive effect.

Results

Individual CeMbio members are detrimental to *C. elegans* survival when compared to *E. coli* OP50, but not when compared to the CeMbio community

Survivorship assays were used to measure the health of lab-adapted (N2) and natural (MY316, MY2768, MY2769) *C. elegans* strains to investigate the impacts of individual CeMbio members and the CeMbio community. Initially, comparisons of these effects were made to *E. coli* OP50 as it is the standard bacterial treatment in the *C. elegans* research community.

When compared to *E. coli*, nearly all individual strains and the microbiome community resulted in decreased survivorship (Fig. 1a, Supplementary Fig. S1 online). This was the case for all *C. elegans* genotypes. The few exceptions to this trend included *Shingobacterium multivorum* BIGb0170, *Comamonas piscis* BIGb0172, and *Ochrobactrum vermis* MYb71. *S. multivorum* had beneficial effects on the health of the MY316 and MY2768 genotypes, and *O. vermis* was beneficial for MY316. Across all nematode genotypes, *C. piscis* had similar effects as *E. coli* OP50. Although comparisons to *E. coli* are common in the *C. elegans* literature, they may not be the most informative when analyzing the effects of microbiome members.

To examine the individual effects within a community context, nematode survivorship on the individuals was also compared to the CeMbio experimental microbiome community (Fig. 1b, Supplementary Fig. S1 online). This resulted in similar significant differences for all genetic backgrounds on the individual strains.

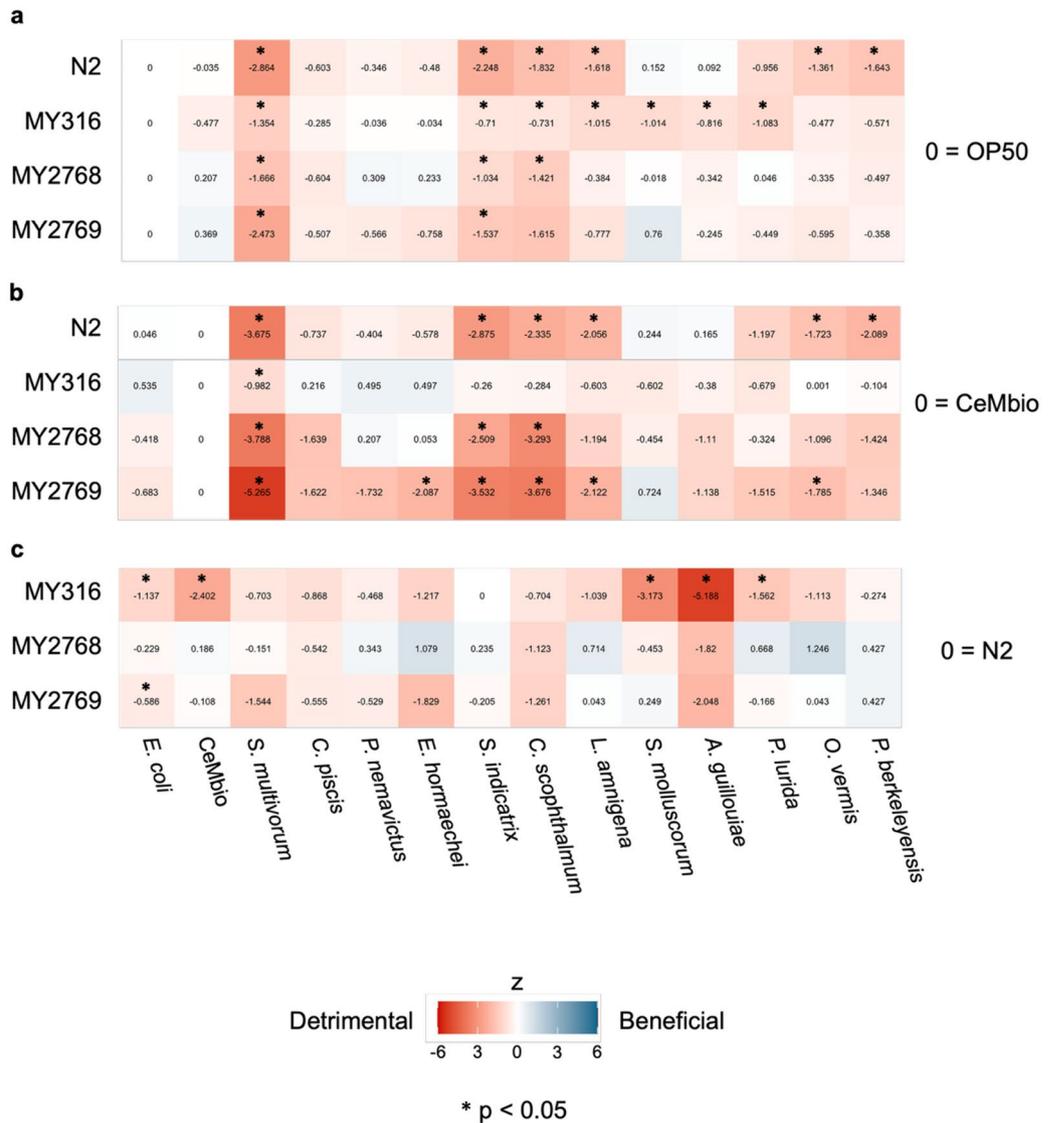


Fig. 2. *C. elegans* fecundity is affected by some individual microbiome strains when compared to CeMbio and *E. coli* OP50. **(A)** Effects of individual CeMbio strains and the CeMbio community on *C. elegans* fecundity compared to *E. coli* OP50. **(B)** Effects of individual CeMbio strains on *C. elegans* fecundity compared to the CeMbio community. **(C)** Effects of individual CeMbio strains and the CeMbio community on fecundity of natural *C. elegans* strains (MY316, MY2768, MY2769) compared to lab adapted N2. For each nematode: treatment combination $n = 10\text{--}60$ (see Methods).

S. multivorum and *C. piscis* were again exceptions to the overall trend. Survivorship of the natural and more recently isolated *C. elegans* strains (MY316, MY2768, MY2769) were significantly reduced when exposed to these two individuals compared to CeMbio. However, survivorship of N2 was increased when exposed to these two strains. N2 survivorship was either not changed or increased when exposed to the individual strains compared to the community with few exceptions. These exceptions include *Enterobacter hormaechei*, *Lelliottia amnigena*, and *Pseudomonas lurida*. The natural genotypes had fewer significant comparisons.

We also compared survivorship of the various nematode genotypes to uncover possible genetic differences in response to these bacterial treatments (Fig. 1c, Supplementary Fig. S1 online). Not many significant differences were observed with the natural nematode strains when compared to the lab-adapted N2 strain. MY316 and MY2768 survived longer on *O. vermis* and MY2769 experienced a decrease in survivorship as compared to N2.

Compared to the whole community, individual members of the microbiome can have beneficial, detrimental, or similar effects on host survival. Based on these observations, there appeared to be combinatorial effects occurring in the microbiome community resulting in the various effects seen by the effects of individual microbiome members. Additionally, host genetics did not appear to play a significant role in the response to individual microbiome members or the community itself. While several specific nematode-microbe combinations were significantly different from the baseline comparisons, we did not observe clear trends that would indicate a specific host genotype was responsible for the observed responses.

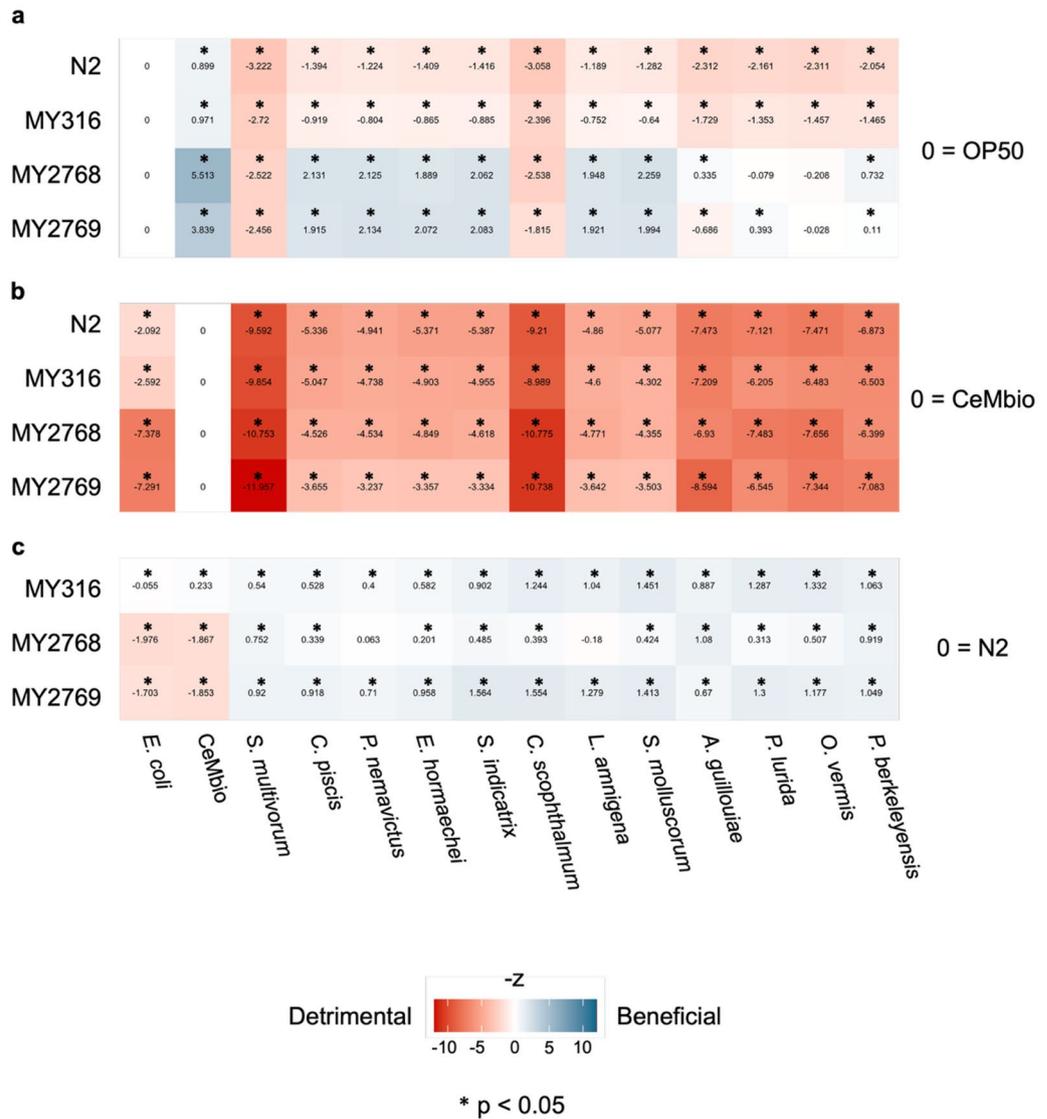


Fig. 3. Individual microbiome members cause slower development than the CeMbio community. **(A)** Effects of individual CeMbio strains and the CeMbio community on *C. elegans* development time compared to *E. coli* OP50. **(B)** Effects of individual CeMbio strains on *C. elegans* development time compared to the CeMbio community. **(C)** Effects of individual CeMbio strains and the CeMbio community on development time of natural *C. elegans* strains (MY316, MY2768, MY2769) compared to lab adapted N2. For each nematode: treatment combination $n=91-1588$ (see Methods).

Fecundity remains unaffected or is decreased when exposed to the microbiome and its individual members

Fecundity was one of the two measures of nematode fitness. The effects of individual microbiome members and the microbiome community on fecundity were analyzed using the same comparisons as for survivorship and visualized using z-scores.

The total number of progeny was similar across most individual bacterial strains when compared to both OP50 and CeMbio (Fig. 2a-b, Supplementary Fig. S2 online). A few individuals caused decreased fecundity in all nematode backgrounds: *S. multivorum*, *S. indicatrix*, and *C. scophthalmum*. Additionally, *L. amnigena* resulted in decreased fecundity for N2, MY316 and MY2769. A few other individual CeMbio members resulted in decreased fecundity but did not result in a specific trend across genotypes. When fecundity was impacted by the bacterial treatment, it always resulted in fewer progeny. Finally, only MY316 was significantly less fecund than N2 (Fig. 2c, Supplementary Fig. S2 online). The fecundity of the other natural strains was similar to N2. Interestingly, the individual microbiome member which resulted in the highest fecundity was always similar to the community, regardless of host genotype. This is similar to previous observations of the effect of prey richness on *C. elegans* intrinsic growth rate³².

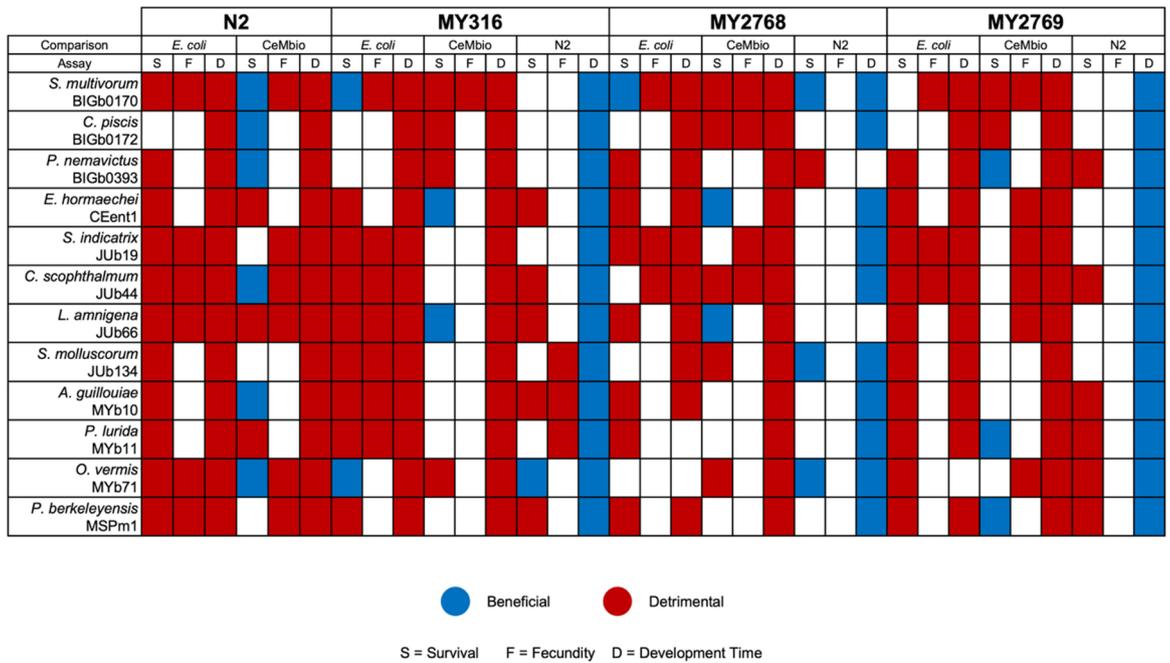


Fig. 4. Summary of individual CeMbio strains effects on *C. elegans* survival and fitness. Overview of the effects of the CeMbio members on *C. elegans* survivorship, fecundity, and development time. The data are divided by nematode genotype, and by the comparison being made (*E. coli* OP50, CeMbio, or N2). Further division is by the type of experiment. Areas without a color signify no significant difference.

Monoculture bacteria cause nematodes to develop slower than the cembio community

Development time was also used as a measure of fitness since it is a major component of generation time that contributes to intrinsic growth rate but is more easily assayed than generation time. Like prior experiments, comparisons were made to both *E. coli* OP50 and CeMbio. A linear mixed model and a general linear hypothesis test was used to determine significance between treatments and between nematode genotypes.

When compared to either OP50 or CeMbio, the individual microbiome members caused significantly different development times (Fig. 3a,b, Supplementary Fig. S3 online). For N2 and MY316, the worms developed faster when exposed to OP50 and CeMbio compared to all individual bacterial strains. MY2768 and MY2769 developed slower when exposed to the individual strains than they did on CeMbio. However, development time of MY2768 and MY2769 was more variable than N2 when exposed to individual CeMbio strains as compared to OP50. The MY2768 and MY2769 worms developed faster when exposed to *C. piscis*, *P. nemavictus*, *E. hormaechei*, *S. indicatrix*, *L. amnigena*, *S. molluscorum*, and *P. berkeleyensis*. For all worm strains, *S. multivorum* and *C. scophthalmum* resulted in significantly slower development when compared to both OP50 and CeMbio. In addition, the natural worm strains generally developed slower than N2 (Fig. 3c, Supplementary Fig. S3 online). Overall, worms reached adulthood faster on a microbial community than they did when exposed to an individual bacterial strain. This suggests that in the community, the individual CeMbio members cooperate to support faster nematode development. Initial characterization of CeMbio found that only two microbiome strains resulted in slower development time compared to *E. coli* and CeMbio²⁰. However, most strains were either similar to or caused faster development compared to *E. coli* and CeMbio. One possible reason for the differences is the temperature at which the experiments were conducted.

Host fitness is influenced by host genetics and bacterial stimuli

Fecundity and development time were both seen to be affected by nematode genotype and bacterial treatment. By investigating the effects seen on different aspects of host fitness, we can investigate the potential fitness strategies organisms use when confronted with different bacterial environments. Although both fecundity and development time were affected, there were comparative differences in how they were affected. This can be visualized by the changes in rank order of individual bacterial effects on fecundity and development time (Supplementary Figs. S2 and S3 online) The bacterial treatments were placed in rank order based on the effects on *C. elegans* N2. For instance, *Lelliottia amnigena* is ranked last in Supplementary Fig. S3 online for development time, indicating that it took the N2 worms the longest when exposed to this bacterium to reach adulthood. However, for fecundity, *L. amnigena* is ranked eighth out of the twelve CeMbio strains, indicating that while N2 fecundity is decreased when confronted with this treatment, it is not the most severe effect. *Shingomonas molluscorum* resulted in the most progeny of the experimental microbiome members for N2, but its effect on N2 development time was the third slowest out of the twelve individual strains.

We can also see that nematode fecundity is often less influenced by the bacterial environment compared to development time (Fig. 4). While the effects of individual CeMbio members on either fitness measure is negative

as compared to the community, the comparative degree of the negative effect is not always the same, visualized by differences in the rank orders of the effects. This suggests differences in host fitness strategies. Additionally, we can see the effects of nematode genotype on aspects of fitness in Fig. 4. Development time is significantly impacted by host genetics, whereas fecundity is less so. These findings further suggest that the host may respond differently to cope with the same environment, and that host genetics influences these mechanisms.

Discussion

Dissection of host-microbiome interactions into host-microbe interactions, as well as microbe-microbe interactions is imperative for a comprehensive understanding of microbiome function. We utilized the model organism *C. elegans* to investigate the roles of individual microbiome members within the community as well as to understand their interactions with a host. The experimental *C. elegans* microbiome, CeMbio, was developed for this purpose²⁰. Here, we have extended the characterization of that tool by determining the effects of individual microbiome members as well as the complete community on host health and fitness. We used survivorship as a measure of health with fecundity and development time as measures of fitness and discovered combinatorial effects of individual members on nematode survival and fitness. This work provides a more complete frame of reference for future studies using CeMbio to investigate host-microbiome interactions.

E. coli OP50 is used by *C. elegans* researchers as the standard comparison for most bacterial treatments^{33–35}. We found that nearly all individual CeMbio members are detrimental to *C. elegans* survival when compared to *E. coli*. This may seem surprising because microbiomes are usually associated with positive health effects. However, similar findings have also been recently documented²³. We also found that the effects of the individual members on *C. elegans* survival could be more beneficial, more detrimental, or similar to that of the CeMbio community. Some individuals such as *Pantoea nemavictus* BIGb0393, *Enterobacter hormaechei* CEent1, and *Lelliottia amnigena* JUb66 were more beneficial for *C. elegans* survival than the microbiome. Others were more detrimental than CeMbio, including *Spingobacterium multivorum* BIGb0170, *Comamonas piscis* BIGb0172, and *Ochrobactrum vermis* MYb71. However, many individual bacterial strains did not affect survivorship significantly. This suggests that when investigating effects of individual microbiome members, comparisons to the microbiome reveal more about individual host-microbe interactions than those compared to *E. coli* OP50.

We also analyzed the effects of individual microbiome members on *C. elegans* fitness components, fecundity and development time. All the significant effects we observed on fecundity were negative. For example, *Spingobacterium multivorum* BIGb0170, *Stenotrophomonas indicatrix* JUb19, *Chryseobacterium scophthalmum* JUb44, and *Lelliottia amnigena* JUb66 reduced fecundity in at least three out of the four genotypes. Development time, however, was significantly lengthened when worms developed on the individuals compared to CeMbio. This was also true for most strains when compared to *E. coli*. Prior research has also documented that worms grown on a monoculture lawn take longer to reach adulthood than those that were raised on a microbial community²⁰. An overview of the effects on both nematode survival and fitness is presented in Fig. 4.

We found that the individuals act combinatorially to influence host survivorship and fitness. As mentioned previously, compared to *E. coli* nearly all the individual members are detrimental to nematode survival. However, when compared to CeMbio, some individual strains resulted in shorter survivorship, but most were either beneficial or not different from CeMbio. This supports the idea that there is a combinatorial effect between the twelve strains that results in the overall effect of CeMbio on *C. elegans* survivorship. This is similar to previous observations where the effects of combinations of microbes on *C. elegans* survivorship were best explained by the “field average” of the effects of individual strains³². Worms that develop on monoculture lawns reach adulthood slower than those exposed to a microbial community suggesting that in the community, individual microbes have complementary properties that can support faster development. Metabolic synergy between the microbes and between the microbes and their host has been shown to influence microbiome composition and assists in maintaining homeostasis^{36–42} and may be one mechanism that contributes to complementarity.

A prior study that examined the effects of individual and combinations of microbes on *C. elegans* fitness found similar results³². That study measured the effects of six individual and all possible combinations of microbes on *C. elegans* reproductive output and generation time to calculate intrinsic growth rate. Darby and Herman found that the effect on intrinsic growth rate was best described by a model of the “best of what’s around”, with some evidence of complementarity that was primarily driven by effects on generation time. The number of microbiome members in the current study did not lend itself to a similar thorough examination of the effects of all possible combinations, nor measurement of generation time. However, the effects we observed on development time, a major component of generation time, are consistent with those prior observations.

Recently Gonzalez and Irazoqui (2024) also found that individual CeMbio members caused a decrease in host survivorship when compared to *E. coli* OP50. They also found that the environment plays a role. Specifically, a richer substrate, tryptic soy agar (TSA), results in decreased survivorship of worms on *E. coli* OP50 than does nematode growth media (NGM), as used in this study. This might account from some differences in the studies such as our finding that *P. lurida* MYb11 was not as detrimental as they demonstrated. Interestingly, they found differential activation of host innate immunity in response to specific CeMbio strains. Furthermore, it appears host genetics also plays a role, in that inactivation of specific *C. elegans* innate immune pathways can cause some CeMbio members to become more virulent than observed in intact animals. However, this may also indicate that *C. elegans* uses innate immune pathways to manage its microbiome. We also examined the influence of host genetics on health and fitness by examining the responses of natural *C. elegans* strains in addition to N2 when exposed to microbiome members or the microbiome community. We also found that host genetics plays a role in this response, especially on host health. Together, these studies suggest that natural genetic variation could exert an effect on the expression of innate immune pathways in response to individual microbiome members. Future work investigating the role of innate immune pathways in the response of natural *C. elegans* to individual

microbiome members as well as to the intact microbiome community may help illuminate the mechanisms that influence host response to the microbiome.

As described in this study, not all microbiome members are beneficial to the host in monoculture. This is also true for humans, though correlation versus causation can be difficult to disentangle. Some examples include *Fusobacterium nucleatum*, *Helicobacter pylori*, and *Eggerthella lenta*, among many others. All three microbes are common in the human microbiome, but have been associated with diseases, including several types of cancer and gut disease^{43–51}. Future studies should also use *C. elegans* and CeMbio to understand the roles of such microbiome members. In a healthy community, the individuals work combinatorially. In a dysbiotic system, the individuals do not work together in the same way as they do when associated with a healthy host. It is also possible that changes in host health status, such as occurs during aging, can impact interactions among microbiome members and relative abundance of members that contribute to dysbiosis. The twelve CeMbio strains are an ideal way to start understanding the role of “detrimental” microbes, or pathobionts, in a microbiome and how they influence host health and fitness when in a community setting. Future work utilizing CeMbio could also aid in describing the mechanisms of these interactions and will increase our knowledge of how specific microbiome members influence host health, including that of humans.

Conclusions

Our study began uncovering the roles of individual microbiome members in the model organism *Caenorhabditis elegans*. Investigations into interactions between the individual microbiome members and the host suggests that the microbiome members act in combination to influence host health. Host fecundity was impacted differently than host health. The microbiome always resulted in a similar number of progeny as that of the best individual microbe. Additionally, if there were significant differences in host fecundity compared to the whole microbiome, it was always detrimental. The final measure of host fitness, development time, suggested complementary effects among microbiome members. Overall, we discovered there are potentially different mechanisms resulting in differences in how host health and fitness respond to individual microbiome members, as well as the whole microbiome. Further work on this topic could lead to understanding how members of a microbiome are associated with host health and disease, including in humans. This work is just the start of understanding individual microbial species impact on host health and fitness.

Data availability

All data generated or analyzed during this study are included in this published article.

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Author contributions

Experimental design of the study was conducted by AF and MH. Experiments and data analyses were performed by AF. Manuscript was drafted by AF. AF and MH read, revised, and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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