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Food, pathogen, signal

The multifaceted nature of a bacterial diet

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C. elegans, both in the wild and in the lab, live on a diet of live bacteria. The bacterial diet provides nutrients for *C. elegans*, but can also play a number of other roles in *C. elegans* physiology. Recently, we compared the effects of different bacterial diets on life history traits and gene expression. Here, we discuss our recent findings in the context of other dietary studies and highlight challenges in understanding dietary effects. For instance, since bacteria can be pathogenic it can be difficult to disentangle pathogenic from dietary effects. Here we summarize different bacterial diets used for *C. elegans* and how they affect the animal.

Like all animals, *Caenorhabditis elegans* has to eat to obtain nutrients for biomass generation during development, growth, and reproduction, as well as to supply energy for basic cellular and organismal processes. *C. elegans* is found all over the world in temperate climates and feed on bacteria that grow on rotting vegetation. When Sydney Brenner first introduced *C. elegans* as a model system he propagated them on *Escherichia coli* OP50, a uracil auxotroph that is growth-restricted by a limited supply of uracil in the media. Because of its limited growth, this strain was more suitable than other bacterial diets for the visualization of worms by microscopy.¹ In recent years, the number of bacterial diets used to propagate *C. elegans* has been expanded and with this expansion we have gained a greater appreciation for the contribution of diet to *C. elegans* physiology.

Bacterial Diets Affect *C. elegans* Life History Traits

Many different bacterial diets can support the development of *C. elegans*. However, physiological characteristics that determine the survival of animals over time, collectively termed “life history traits,” may be affected differently by different diets. These traits include lifespan, fertility, and developmental rate²⁻⁵ (Table 1). For example, animals fed *E. coli* HB101 or *Comamonas* DA1877 develop faster than animals fed *E. coli* OP50 or *E. coli* HT115.^{3,6} Diet-induced phenotypic effects are accompanied by dramatic differences in gene expression,^{2,3} metabolic profile,⁷ and fatty acid composition.⁸ It is likely that bacteria exert their effects in a number of ways; through differences in nutritional quality, production of signaling molecules, pathogenic effects, their suitability for consumption by *C. elegans*, or through as-yet-unidentified mechanisms (Fig. 1). An emerging theme in *C. elegans* research is to elucidate how diet induces molecular changes and how these changes affect the animal.

Components of a Bacterial Diet: Macronutrients and Micronutrients

Bacterial diets supply macronutrients such as carbohydrates, fats, and proteins that are needed to make biomass during growth and reproduction and to generate energy. In addition, bacteria provide essential micronutrients such as vitamins and co-factors. A first question related to how bacterial diet affects the worm is whether

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Table 1. Reported effects of bacterial diet on *C. elegans*. Included are effects described for N2 grown on NGM, relative to *E. coli* OP50 unless otherwise specified

| Strain | Effect on <i>C. elegans</i> | Reference |
|--------------------------------------|--|-----------|
| <i>Bacillus cereus</i> S13 | Decreased growth rate* | 4 |
| <i>Bacillus licheniformis</i> S3 | Decreased growth rate* | 4 |
| <i>Bacillus megaterium</i> | Increased lifespan | 2 |
| <i>Bacillus megaterium</i> L10 | Decreased growth rate* | 4 |
| <i>Bacillus sp</i> S9 | Decreased growth rate* | 4 |
| <i>Bacillus subtilis</i> | Increased lifespan | 22 |
| <i>Comamonas</i> DA1877 | Increased quiescence* | 23 |
| <i>Comamonas</i> DA1877 | Decreased lifespan | 3 |
| <i>Comamonas</i> DA1877 | Decreased brood size | 3 |
| <i>Comamonas</i> DA1877 | Accelerated growth | 3 |
| <i>Comamonas sp</i> H39 [†] | Accelerated growth* | 4 |
| <i>Escherichia coli</i> HB101 | Decreased fat storage—smaller lipid droplets, reduced triacylglycerol levels | 8 |
| <i>Escherichia coli</i> HB101 | Increased quiescence* | 8 |
| <i>Escherichia coli</i> HB101 | Accelerated growth* | 2 |
| <i>Escherichia coli</i> HB101 | Accelerated growth | 4 |
| <i>Escherichia coli</i> HT115 | Decreased fat storage—smaller lipid droplets, reduced triacylglycerol levels | 2 |
| <i>Micrococcus luteus</i> | Decreased lifespan | 4 |
| <i>Pantoea dispersa</i> W8 | Accelerated growth* | 4 |
| <i>Pseudomonas sp</i> | Increased lifespan | 8 |
| <i>Pseudomonas sp</i> B7 | Accelerated growth* | 8 |
| <i>Pseudomonas sp</i> W11 | Accelerated growth* | 2 |

*Compared with DA837, a streptomycin-resistant isolate of *E. coli* OP50; [†]*Comamonas sp.* H39 is the parent strain of *Comamonas* DA1877.

gross differences between macronutrients occur between bacterial foods and, if so, whether these differences can explain changes in life history traits. Gross analysis of bacterial carbohydrates, lipids, and proteins has revealed that bacterial strains can differ in their macronutrient content. For example, *E. coli* HB101 and *E. coli* HT115 contain three to five times the amount of total carbohydrate found in *E. coli* OP50.⁸ We recently asked whether differences in macronutrients or caloric content between *E. coli* OP50 and *Comamonas* DA1877 could explain the dramatic effects on worm life history traits, notably the accelerated development on the latter diet. We first measured bulk carbohydrate, protein and fat levels in each bacterial species and found that *E. coli* OP50 and *Comamonas* DA1877 do not differ significantly in the overall levels of these macronutrients.³

Importantly, we found that even small amounts of *Comamonas* DA1877 mixed into the *E. coli* OP50 diet could accelerate growth and elicit similar changes in gene expression, demonstrating that it is not the bulk levels of macronutrients that explain these differences. Thus, there is much more to consider in a bacterial diet than simply gross macronutrient levels and/or caloric value.

In addition to differences in macronutrients, bacteria likely also differ in the repertoire and amounts of micronutrients they produce. Micronutrients are defined as compounds that are present in small amounts, including trace elements and vitamins that can act as essential metabolites and co-factors. The presence or absence of these micronutrients has the potential to significantly influence metabolic pathway usage and, thus, result in

systemic changes. For example, folate, as tetrahydrofolate, acts as a substrate for many single carbon transfer reactions. Thus, altering levels of folate has the potential to change metabolic pathway usage and alter *C. elegans* physiology resulting in phenotypic consequences. In fact, high levels of folate have a negative impact on lifespan, as reducing folate levels in the *E. coli* diet by mutation of the *aroD* gene extends lifespan.⁹

One caveat in measuring overall levels of nutrients is that although different strains of bacteria may be similar in dietary content, the ability of *C. elegans* to ingest these bacteria may alter the availability of nutrients for the animal. It has been proposed that size and stickiness (resulting from clumping of bacteria) may affect the ability of bacteria to be ingested.⁴

Bacterially Derived Signals

Bacterially derived factors may affect *C. elegans* in a number of ways. Some may act as nutrients, contributing to the generation of biomass or energy, while others may act as chemical signals acting, either positively or negatively, on cellular processes. This is perhaps best studied with respect to pathogenic strains of bacteria, from which some bacterially derived signals stimulate an immune response and, thus, benefit the worm while others act detrimentally by interfering with the innate immune response.¹⁰ In addition, some dietary molecules may act as signals that divulge information about the environment. A number of bacterially derived molecules are neuronally sensed by *C. elegans* and result in attractive or repulsive behaviors (for a review, see ref. 11). These sensing mechanisms are likely used to identify suitable food sources and avoid harmful environments.

E. coli produces more than a thousand metabolites and small molecules.¹² A complete metabolomic analysis of all bacterial species and strains that can be used as a *C. elegans* diet has yet to be accomplished. However, there is no doubt that the complement of molecules produced differs between bacterial species and strains. Among the plethora of molecules produced by bacteria, there are likely to be a number of metabolites that function as hormones or signaling molecules for the worm. For example, *C. elegans* cannot synthesize nitric oxide (NO), as they lack nitric oxide synthase (NOS). However, bacterially produced NO can act as a potent signaling molecule in the worm, resulting in extended lifespan.¹³ In another study, metabolites derived from bacterial tryptophan metabolism were shown to mitigate diet-dependent sterility in animals that carry a mutation in a specific nuclear hormone receptor.¹⁴ Longer term, it will be important to identify all bacterial molecules that can affect *C. elegans* life history traits, as well as the mechanisms involved.

Challenges of Using a Live Diet

C. elegans is unique among model organisms in that animals are propagated

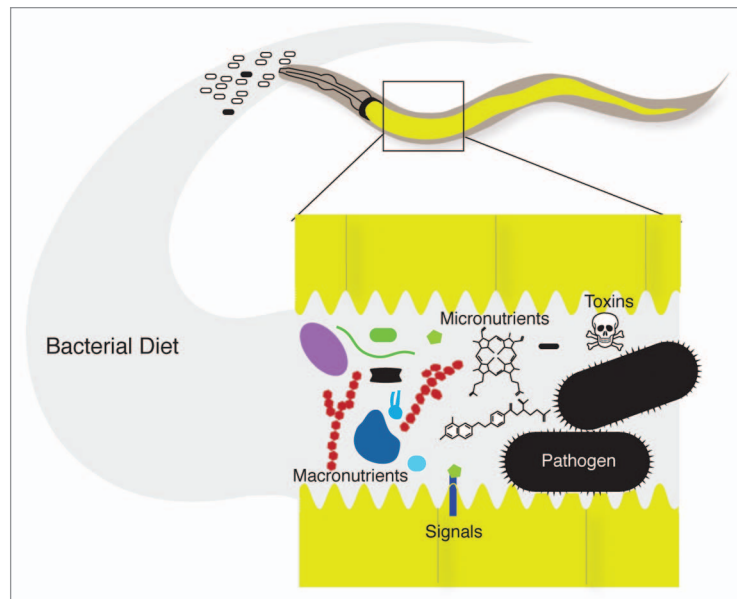


Figure 1. The bacterial diet includes macronutrients, such as proteins, lipids and carbohydrates, micronutrients, and a plethora of molecules that may function in a number of different ways as signals, hormones, or toxins. In addition, bacteria may have pathogenic effects.

on a live diet. While historically this has been based on convenience, there are additional advantages. For example, as mentioned above, live bacteria may serve as convenient delivery systems for nutrients or other biomolecules that are short-lived (e.g., NO). The presence of growing bacteria may guarantee the continued synthesis of these molecules, and, thus, the availability of these factors throughout the life of the animal.

C. elegans has been extensively used to model the effects of pharmaceutical drugs and to identify genetic factors involved. One concern in using live bacteria is the potential for those bacteria to metabolize and modify drugs or other factors that are added to the diet. Bacterial metabolism may alter or inactivate specific drugs or dietary factors. In addition, factors may exert their effects indirectly on the worm by affecting the bacteria. For instance, the anti-diabetic drug metformin extends lifespan in *C. elegans* only when animals are grown on live bacteria.¹⁵ This strongly suggests that this life extension is not due to metformin itself, but occurs in response to a bacterial factor that is affected by metformin, or to a metabolized form of the drug. In fact, the authors demonstrate that the lifespan-extending effect of metformin occurs by altering bacterial folate metabolism.

A final consideration for a live diet is the potential for genetic drift of bacterial strains between labs. The potential for bacteria to acquire mutations may result in different labs observing different effects for what is believed to be the same bacterial strain. This has been proposed to explain lab-to-lab variation in *C. elegans* lifespan fed the “same” bacterial strain.² The potential for bacterial mutation is illustrated in a study aimed at identifying mediators of aging using an RNAi feeding strategy.⁹ For one life-extending clone, the effect persisted even after the RNAi plasmid was lost. It was found that a mutation in the *E. coli* HT115 strain that resulted in the inactivation of the *aroD* gene, which is involved in folate synthesis, was responsible for the effect on lifespan.

Pathogenic Effects of Diet

In addition to providing a source of nutrients, live bacteria can also present a challenge to the animal, as many can be pathogenic. In 1999, Fred Ausubel’s group described the use of *C. elegans* as a model for pathogenesis using the broad range pathogen *Pseudomonas aeruginosa* PA14, which kills animals within 3 days.¹⁶ Subsequently, the number of bacterial species classified as *C. elegans* pathogens was expanded and a number of studies

sought to identify and characterize bacterial factors responsible for pathogenicity (for a review, see ref. 17). In addition, a number of gene expression studies sought to identify pathogenic response factors. One consideration in the interpretation of these experiments is the fact that, in these studies, pathogen also served as food and, thus, effects due to pathogenic processes may be entangled with those resulting from dietary factors.

Recently, we identified a set of dietary response genes that differed in expression between animals grown on two non-pathogenic strains, *E. coli* OP50 and *Comamonas* DA1877.³ In addition, we demonstrated that many of these genes are also responsive to metabolic perturbation.¹⁸ Among these genes, *acdH-1* showed the largest change in expression between the two diets, being dramatically down-regulated on *Comamonas* DA1877 compared with *E. coli* OP50. Intriguingly, *acdH-1* is also reduced in response to a number of pathogens, including *P. aeruginosa*,¹⁹ *S. aureus*,²⁰ *E. carotovora*, *E. faecalis*, and *P. luminescens*.²¹ In all cases, these pathogens were compared with *E. coli* OP50. This raises the possibility that some of the effects of pathogenic bacteria on gene expression may be dietary rather than pathogenic. Indeed, we observed that changes in gene expression reported for animals exposed to pathogenic *P. aeruginosa* overlapped significantly with changes in expression observed on animals fed non-pathogenic *Comamonas* DA1877.³

C. elegans as a Model for Microbiota Effects on Mammals

The diverse bacterial community inhabiting the human intestine is known as the gut microbiota. These bacteria are critically important for health as they aid digestion, synthesize essential compounds, and play important roles in immunity to enhance resistance to pathogens. Because bacterially derived compounds can profoundly affect *C. elegans*, it is tempting to speculate about the use of *C. elegans* as a model to study how gut microbiota affect human health. However, it is not clear whether bacteria truly function similarly in worms because it has yet to be determined whether *C. elegans* has a healthy, living microbiota

in its natural environment. Nevertheless, *C. elegans* have an intimate relationship with their food because they live in it and are therefore constantly exposed to bacterial metabolites. Thus, for *C. elegans*, food may have similar effects as the microbiota in humans even before bacteria colonize the gut. The fact that *C. elegans* can be fed different bacteria and that bacteria can profoundly affect the animal's life history traits, combined with the genetic tractability of both worms and bacteria indicates that worms will continue to be a powerful model not only for development and aging, but also for dietary and microbiota effects in humans.

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