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Article

Comparing the dietary preference of *Caenorhabditis elegans* for bacterial probiotics vs. *Escherichia coli*.

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SUMMARY

The use of probiotics is on the rise as more people are learning about their possible health benefits. In this experiment, we used *C. elegans* as a simple model organism to observe the impact of probiotics on the human digestive system. In this investigation the dietary preference of *C. elegans* was tested using three different bacterial probiotics (Chobani, siggi's and Stonyfield) and the control nutrient source, *E. coli*. The results of the experiment showed that the *C. elegans* were, on average, most present in Chobani cultures. At the end of the observation period, there were, on average, about 1,207 *C. elegans* present in the *E. coli* quadrants, and about 1,314 *C. elegans* present in the Chobani quadrants, as compared to 1,133 *C. elegans* in the siggi's quadrants and 981 in the Stonyfield quadrants. There were about 8.9% more *C. elegans* present in the Chobani quadrants than in the *E. coli* quadrants. Additionally, the Chobani quadrants grew, on average, by 188 *C. elegans*/day, 9.3% more than the 172 *C. elegans*/day growth in the *E. coli* quadrants. While not statistically significant, these results still demonstrated that *C. elegans* might prefer Chobani cultures over other probiotic yogurts, which may also indicate greater gut benefits from Chobani over the other yogurt brands tested.

INTRODUCTION

For decades, the yogurt aisle in American supermarkets had been dominated by corporations like General Mills and Kraft (1). Against all odds, a Turkish immigrant named Hamdi Ulukaya brought his Greek yogurt, Chobani, to the United States in 2007 (2). Detractors were skeptic that a yogurt with live bacteria cultures would sell due to public discomfort around eating bacteria (3). However, the public was convinced with Chobani's health benefits - twice as much protein as regular yogurts, no artificial sweeteners, and 3 probiotics (4). Today, Chobani pulls over $1.5 billion in annual revenue is the second largest yogurt titan in the US and has inspired other companies to increase the nutritional value of their own yogurts (5). Like Greek yogurt, Icelandic yogurt ("skyr"), including the brand siggi's, is strained and provides a similar protein content, low sugar level, and number of probiotics (6).

Probiotics are microorganisms that are present in Greek and Icelandic yogurts, as well as in American yogurts like Stonyfield today (7). The concept behind probiotics was introduced in the early 20th century, when Nobel Laureate Elie Metchnikoff, known as the "father of probiotics," proposed that consuming beneficial microorganisms could improve people's health (8). Researchers continued to investigate this idea, and the term "probiotics" - meaning "for life" - eventually came into use. Probiotics can have various health benefits, such as enhanced digestion and immune function in humans (9-10). Products with probiotics include foods like yogurt, dietary supplements, and even skin creams. The use of probiotics has been on the rise in the last decade, as more people are learning about their health benefits (11). The probiotic market globally is worth over $15B USD due to increasing research of their health benefits and efficacy in treating certain diseases (12). While Chobani, siggi's, and Stonyfield share some strains of probiotic bacteria, they also have probiotics unique to their preparations (**Table 1**) (7, 13-14).

In this experiment, we aimed to see the impact of these different yogurt preparations on humans by using the model organism *Caenorhabditis elegans,* a nematode roundworm that is around 1 mm long (15). Our goal was to see whether probiotics have a positive effect on the growth of *C. elegan s*, which normally feed off and grow in decomposing plants which are rich in bacteria (16). In the lab, *C. elegans* are often fed *Escherichia coli*, so we chose it as our control food source (16). Even though they are much smaller than humans, many of their organ systems are like those of humans and other mammals, making *C. elegans* a good model organism for our study (17). Since both *C. elegans* and humans share similar digestive tissue, we aimed to determine the effect of probiotics on the growth of *C. elegans* to possibly infer outcomes within the human gut, although we cannot determine exact outcomes unless specifically tested on humans (17). In addition, *C. elegans* was chosen as the model organism because they are easy to culture and use, which made them well suited for this type of independent study.

Table 1. Number and strains of probiotic cultures in each yogurt.

Yogurt	Probiotics and Active Cultures	Number of Cultures
Y1 (Chobani)	*S. thermophilus, L. bulgaricus, L. acidophilus, Bifidus, L. casei*	5
Y2 (siggi's)	*S. thermophilus, L. delbrueckii subsp. bulgaricus, B. lactis, L. acidophilus, L. delbrueckii subsp. lactis*	5
Y3 (Stonyfield)	*S. thermophilus, L. bulgaricus, L. acidophilus, Bifidus, L. paracasei, L. rhamnosus*	6

Note: Cultures identified from the nutrition facts provided by each company (7, 13-14).

A culture of probiotics was grown for each of the three yogurt brands to become a food source for the *C. elegans*. To limit sources of variability, each of the three yogurts were plain with 0% milkfat. By seeing which probiotic or *E. coli* the *C. elegans* population grows most in, we inferred which culture might be most beneficial for growth.

Since Stonyfield yogurt has the largest variety of active cultures, we hypothesized that *C. elegans* would migrate to areas with Stonyfield as there would be a greater chance that *C. elegans* would prefer eating at least one of the various active cultures and potentially display the best growth. We also hypothesized that all the probiotic yogurts would promote *C. elegans* growth more than the control group, *E. coli,* due to the availability of additional active cultures. Ultimately, we found that the Chobani fed *C. elegans* had the highest population increase, differing from what we had hypothesized. After Chobani, the control food source (*E. coli*) had the most *C. elegans* growth followed by the siggi's. The areas with Stonyfield yogurt ended up with the least amount of *C. elegans*, on average.

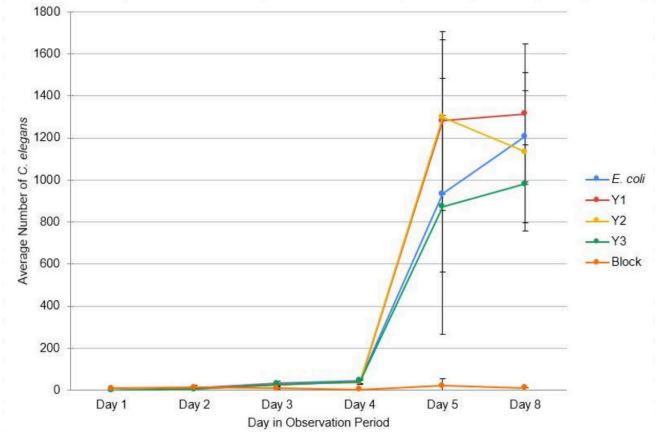

Figure 1. Average number of *C. elegans* each day by food source. Data represent the *C. elegans* population in all quadrants and the central block, as well as the overall population growth. Graph shows the average number of *C. elegans* by food source each day, where different colors represent the food sources. Data was measured daily for 5 days and again on Day 8. The data values are the average number of *C. elegans* in all 8 petri dishes. Error bars represent standard deviation.

RESULTS

The goal of this experiment was to learn more about the effect of probiotics on human nutrition. Today, *C. elegans* is widely used as a model organism to study humans and other mammals (17). We compared *C. elegans*'s growth in their typical laboratory food source, *E. coli*, versus three probiotic yogurts (16).

To study the dietary preferences of *C. elegans*, eight petri dishes were partitioned into four quadrants. The four quadrants were then labeled as E, for *E. coli*, Y1 for Chobani, Y2 for siggi's, and Y3 for Stonyfield. The *C. elegans* were added to the center of each petri dish and were observed daily for 5 days and once more on day 8 of the experiment.

Y1 (Chobani yogurt) had the most *C. elegans* at the end of the observation period, followed by *E. coli* (which acted as the control food source environment), Y2 (siggi's), and finally Y3 (Stonyfield) (**Table 2**). On Day 8, there was an average (across all 8 petri dishes) of 1314 *C. elegans* in the Y1 quadrants, 1207 *C. elegans* in the *E. coli* quadrants, 1133 *C. elegans* in the Y2 quadrants, and 981 *C. elegans* in the Y3 quadrants. The block at the center of each petri dish, where the *C. elegans* were initially placed, contained Luria broth and agar. Since the block is not as nutrient rich as the other food sources, *C. elegans* numbers are consistently low in the block, with an ending average of 12 *C. elegans*, almost identical to its starting value of 10 *C. elegans*.

Table 2. Average number of *C. elegans* per day.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Average in *E. Coli*	0 ± 0	10 ± 5.6	32 ± 11	46 ± 13	934 ± 370	1207 ± 220
Average in Y1	0 ± 0	10 ± 6.0	29 ± 13	38 ± 8.3	1281 ± 420	1314 ± 330
Average in Y2	0 ± 0	6 ± 3.6	26 ± 9.7	40 ± 11	1230 ± 370	1133 ± 380
Average in Y3	0 ± 0	6 ± 1.5	27 ± 17	43 ± 13	874 ± 610	981 ± 190
Average in Block	10 ± 2.1	14 ± 10	9 ± 5.8	3 ± 2.0	21 ± 34	12 ± 0
Average Total Number of *C. elegans* (per plate)	10 ± 2.1	45 ± 16	123 ± 48	170 ± 29	4411 ± 1200	4646 ± 740

Note: Data were measured daily for 5 days, with an extra measurement on Day 8. Values represent the average number of C. elegans *in all 8 petri dishes ± standard deviation. Data not recorded on Day 6 and 7.*

Up until Day 4, the growth of the *C. elegans* population in all quadrants was relatively low (**Figure 1**). While some individual petri dishes had large *C. elegans* population increases before Day 4, others even had decreases in their *C. elegans* population, so the average *C. elegans* population growth was rather low as compared to after Day 4. In the period after Day 4, *C. elegans* population in all quadrants grew rapidly each day. One exception was the average *C. elegans* population change from Day 5 to Day 8 in the Y2 quadrant, where the *C. elegans* population decreased. The average daily increase in the *C. elegans* population was calculated for all quadrants in each petri dish (**Figure 2**).

The quadrant with the highest average daily increase in number of *C. elegans* was the Y1 quadrant, which increased, on average, 188 *C. elegans*/day. This was followed by the *E. coli* quadrant, which increased by an average of 172 *C. elegans*/day. Next was the Y2 quadrant, which increased an average of 162 *C. elegans*/day. The Y3 quadrant had the lowest daily increase of the quadrants at 140 *C. elegans*/day. Finally, the block had the lowest average daily increase, with an increase of 0 *C. elegans*/day, because of its low nutrient levels compared to the quadrants' food sources.

Three paired one-tailed *t*-tests were performed between *E. coli* and each of Y1, Y2, and Y3. However, only Y1 had a higher average daily increase in *C. elegans* than *E. coli*, and Y2 and Y3 had lower increases in *C. elegans* than *E. coli* on average. *E. coli* was compared to Y1, and the result was not statistically significant using a significance cutoff of $\alpha < 0.05$ (*p*-value = 0.19). Since the other quadrants (Y2 and Y3) had lower increases in *C. elegans* than Y1 and even *E. coli*, their results were also not statistically significant (with the alternate

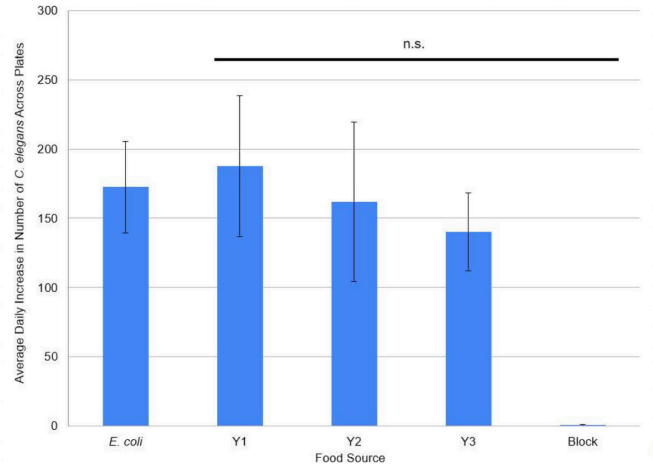

Figure 2. Average daily increase in number of *C. elegans* by food source. Data represent the average daily increase in number of *C. elegans* in each quadrant of the petri dishes/plates. Data was measured daily for 5 days and an extra measurement was done on Day 8. The average was calculated across all eight petri dishes. Error bars represent standard deviation. Not significant, compared to the control group (*E. coli*), is denoted by n.s. (n = 6, paired t-test, $\alpha = 0.05$).

hypothesis that the probiotic quadrants would perform better than the *E. coli* quadrants.

DISCUSSION

Our initial hypothesis was that overall, all probiotic yogurts tested would have a greater increase in number of *C. elegans* than the *E. coli* quadrants. The specific hypothesis was that since Stonyfield (Y3) has the largest variety of active cultures, it would promote the highest population increase in *C. elegans*. Therefore, we hypothesized that the *C. elegans* would consume most of the Stonyfield active cultures and that they would be found the most often in this quadrant compared to the others. However, the experiment did not support the original hypothesis. By the end of the experiment, *C. elegans* had the highest population increase in the Chobani (Y1) quadrants. Therefore, our specific hypothesis that *C. elegans* population would increase the most in Stonyfield quadrants was not observed in this experiment. Our general hypothesis that probiotic yogurt quadrants would promote *C. elegans* growth more than *E. coli* quadrants was also not observed, as we could not reject the null hypothesis.

Although the average daily increase in number of *C. elegans* in Y1 was not statistically significant compared to the *E. coli* quadrants, the Y1 quadrants still had a higher average and overall increase in their *C. elegans* population. The Y1 quadrants increased, on average, by 188 *C. elegans*/day, which is 9.3% more than the 172 *C. elegans*/day increase in the *E. coli* quadrants. Additionally, on Day 8, there was an average of 1314 *C. elegans* in the Y1 quadrants: 8.9% more than the average of 1207 *C. elegans* in the *E. coli* quadrants. This shows that Y1 (Chobani) is still an effective food source for *C. elegans* and could still be more effective than *E. coli*. Further study could validate this result. Additionally, *C. elegans* did not prefer the most diverse food source in terms of active cultures (Stonyfield, Y3). This may indicate that *C. elegans* do not necessarily favor diverse food sources.

Many individual petri dishes indicated a possible food cycle in some quadrants because their numbers of *C. elegans* per day fluctuated, instead of constantly increasing. For example, in the Y2 quadrant of the 6th petri dish, the *C. elegans* population was growing rapidly since the initial observation up until Day 2. There were around 1200 *C. elegans* observed on Day 2, but the population dramatically decreased to 200 *C. elegans* on Day 3, and 20 *C. elegans* on Day 4. This could be because of a food shortage in the 6th petri dish's Y2 quadrant. Since the *C. elegans* population was growing so rapidly, the *C. elegans* population could have reached its carrying capacity in that quadrant, so no food was left, and many *C. elegans* could have died or migrated. Once the probiotic bacteria in Y2 replenished itself due to a low *C. elegans* population on Day 4, the *C. elegans* had enough food to grow once again, so the *C. elegans* population grew to around 2213 on Day 5. Most petri dishes showed patterns that might indicate a food cycle on similar days and, on average, reflected the same trends.

There were several possible sources of error that could have influenced the outcome of this experiment. Some environmental factors could not be controlled. Each petri dish was wrapped with Parafilm because otherwise the *C. elegans* might have left the dishes, since they move so fast and easily. Despite re-wrapping after every data collection, the humidity and the temperature of the room could not be controlled uniformly. This inconsistency could have been a potential cause of variability of *C. elegans* thriving on certain days more than others. Although all the plates were in the same environment, they could have been affected in other ways. Another likely source of error was that the microscope had some limitations. The microscope was not powerful enough to magnify the *C. elegans* enough to see their embryonic and larval stages, possibly giving an inaccurate count of *C. elegans*. An additional factor that could not be controlled was how fast the three yogurt cultures grew and how much of it was eaten. There was no way to tell how much of each nutrient source was available at each time. In addition, each of the three yogurts were a mix of multiple active cultures, meaning that the relative growth of each bacterial strain in the cultures could have impacted the available food sources and reduced the diversity of those cultures. Also, when the *C. elegans* were being placed in each of the 8 petri dishes, they may not have been exactly centered, so the *C. elegans* might have been more inclined to go to a quadrant closest to them. Another source of error could have occurred while counting the *C. elegans* on the petri dishes. Some of the *C. elegans* were clumped together, making it difficult to count them. As a result, this likely caused the numbers of *C. elegans* that were counted to differ slightly from the actual number. *C. elegans* were growing and moving at an accelerated rate, possibly making the counts marginally inaccurate.

This experiment raised multiple questions for further

investigation. One of these questions is testing out other food sources to find out *C. elegans*'s preferences. *C. elegans* 's diet has been studied in other experiments, including a study testing *C. elegans*'preference for two different strains of *E. coli* (18). To continue studying *C. elegans*'s diet, one expansion of this experiment could study how varying amounts of probiotic cultures could affect the growth of *C. elegans*. In this experiment, each yogurt had some of the same active cultures as other yogurts, but mostly differed in their selection of probiotics. It would be interesting to see if preferences change with other probiotic cultures not tested in this experiment. It would also be interesting to see if a higher concentration of probiotic cultures could increase the overall population growth of *C. elegans*. Another area of deeper and further examination would be to grow each culture in its own petri dish and then allow *C. elegans* to consume it individually as opposed to having multiple food sources share a petri dish.

In conclusion, this experiment demonstrated that *C. elegans*' population size grew most effectively in Chobani, possibly indicating that the cultures Chobani contained were the preferred source of nutrition compared to that of other probiotic yogurts and *E. coli*. This result may apply to humans as well due to the shared similarities in the gut with *C. elegans*.

MATERIALS AND METHODS

Culture growth and maintenance

A sterile plastic milliliter pipette was used to transfer four drops of each yogurt sample and the control - Y1 (Chobani), Y2 (siggi's), Y3 (Stonyfield), and *E. coli* (Carolina Biological Supply) - into 6 mL Luria broth (Carolina Biological Supply). A sterile inoculation loop was used to mix each yogurt and *E. coli* with Luria broth until evenly distributed into each test tube. This mixture formed the culture of probiotics for each yogurt brand and for *E. coli*. The test tubes were placed in a room overnight (70°F), and later in the biology classroom (70°F).

Eight petri dishes were then filled with agar. One bottle of agar was melted using a microwave and poured equally into each of the eight petri dishes. They were kept solidifying for the length of the school day (approximately eight hours), before turning them upside down to prevent too much condensation. The petri dishes were then wrapped in Parafilm and stored in the fridge overnight.

The next day, the petri dishes were split into four quadrants (on the outside of the petri dish) by using a marker to equally separate the dishes into four parts. The four quadrants were the three yogurt cultures and one control food source (*E. coli*). Each of the four parts was labeled as E for *E. coli*, Y1 for Chobani, Y2 for siggi's, and Y3 for Stonyfield. Using a micropipette, 200 ul of *E. coli* and each of the three probiotic yogurt cultures from the test tubes were aliquoted and spread with an inoculation loop into their respective quadrants in the eight petri dishes. They were then left to sit for five hours at room temperature (70°F).

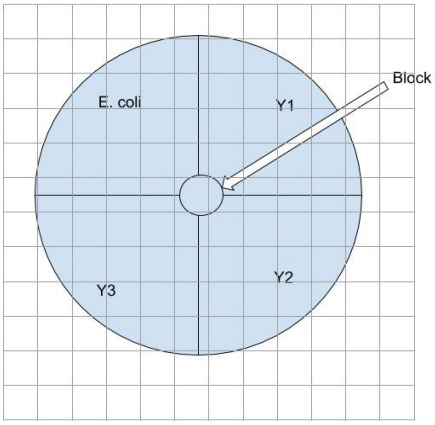

Figure 3. Petri dish setup. Each dish was divided into quadrants and, and graph paper was placed under each dish to approximate the count in each quadrant. *C. elegans* were counted in one square at the center of each quadrant and multiplied by the number of squares per quadrant to estimate the total *C. elegans* in each quadrant.

C. elegans maintenance and observation

The *C. elegans* used in the experiment (Carolina Biological Supply Company) came in a petri dish of agar and Luria broth, and a 1 cm^2 block of *C. elegans* was cut out and placed in the center of each of the eight experimental petri dishes. Each 1 cm^2 block of agar had an average of ten *C. elegans*. The *C. elegans* were first observed 2.5 hours after their initial placement in the center of the experimental petri dishes.

During the first observation, the *C. elegans* were counted once again in each quadrant as well as in the block. We used a microscope with 100-400x magnification to count the number of *C. elegans* throughout the observation period. The *C. elegans* were counted daily for 5 days and once more on day 8 to see the population growth in each of the 4 different food sources, as well as the block.

On Day 3 of the observation, there were too many *C. elegans* in each quadrant for them to be accurately counted, so a piece of graph paper was placed under each petri dish. The *C. elegans* were then counted in one square in the center of each quadrant. These numbers were then multiplied by the number of squares per quadrant to calculate an estimate of how many *C. elegans* were in each quadrant (**Figure 3**). After data collection, daily averages of the number of *C. elegans* in each quadrant (of all dishes) were calculated (**Figure 1**).

Average daily increase calculation

Average daily increases in the *C. elegans* population were then calculated to see which food source *C. elegans* consume most over time and to see in which of the 4 food sources their population grew fastest (**Figure 2**).

For example, in the Y3 quadrant of the second petri dish, the initial observation on Day 1 had 0 *C. elegans,* and the final observation on Day 8 had about 1406 *C. elegans*. Therefore, the average daily increase in *C. elegans* in the Y3 quadrant of the second petri dish was:

$$\frac{Day\ 8\ Population - Day\ 1\ Population}{7\ Days} = \frac{1406 - 0}{7} = 201\ C.elegans/\text{day}.$$

Then, the average across all petri dishes was taken, so the average daily increase for Y3 became 140 *C. elegans*/day (shown in **Figure 2**), because other petri dishes' Y3 quadrants had lower daily increases in *C. elegans* population.

Statistical analysis

Three paired one-tailed *t*-tests were performed between *E. coli* and each of Y1, Y2, and Y3 with a significance cutoff of $p < 0.05$. In this test, the alternate hypothesis was that the probiotic quadrants would perform better than the *E. coli* quadrants, and the null hypothesis was that *E. coli* and probiotic yogurt quadrants would have the same *C. elegans* population increase.

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