

# *P. fluorescens* and *B. megaterium* Effect on the Lifespan of Mutant-Type Dpy-11 and Wild-Type of *C. elegans*

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**How to cite this paper:** Ross, R. (2022) *P. fluorescens* and *B. megaterium* Effect on the Lifespan of Mutant-Type Dpy-11 and Wild-Type of *C. elegans*. *CellBio*, 11, 1-9.  
<https://doi.org/10.4236/cellbio.2022.111001>

**Received:** February 1, 2022

**Accepted:** March 18, 2022

**Published:** March 21, 2022

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## Abstract

The research question being studied in this paper is how do different types of bacteria as food (*Pseudomonas fluorescens* and *Bacillus megaterium*) affect the lifespan of *Caenorhabditis elegans* in dpy-11 mutant-type and wild-type? *P. fluorescens* and *B. megaterium* will be the two pathogens that will be tested on two different types of *C. elegans*: mutant-type dpy-11 and wild-type. From the analysis of primary articles studying these pathogens, it can be concluded that *P. fluorescens* and *B. megaterium* are decent contenders for allowing *C. elegans* to grow and possibly extend the lifespan of it. *P. fluorescens* will allow the lifespan of the two types of nematodes to be longer. Additionally, the mutant-type dpy-11 of *C. elegans* will have a much longer lifespan, even double, compared to that of the wild-type. The results showed *P. fluorescens* had a longer lifespan than *B. megaterium* but not as long as *C. elegans*' main food source, *E. coli*. *C. elegans* mutant dpy-11 had a longer lifespan than the wild-type. Furthermore, there were no *C. elegans* present in the *B. megaterium* wild-type plates.

## Keywords

*C. elegans*, *P. fluorescens*, *B. megaterium*, *E. coli*

## 1. Introduction

*Caenorhabditis elegans*, otherwise known as *C. elegans*, is a free-living nematode worm approximately 1 mm in length that has been used as a model organism for reproduction and nutrition [1]. These near-microscopic worms are usually found naturally in humid patches of soil rich in decaying plant material. In nature, *C. elegans* feed on soil bacteria, rotting fruit, and eukaryotes (mostly yeast) [2]. They are capable of making complex decisions based on the presence and

quality of food in their environment and can seek food that best supports their growth [2].

Free-living species (as opposed to parasitic species) can be cultured in the absence of a host through their whole life cycle, which makes them ideal for observation and allowed for major discoveries in molecular and cell biology. Studies of various free-living nematodes started with Maupas in 1899 and 1900, followed by studies from 1913 to 1925 that focused on reproduction and cytology [1]. *C. elegans* were adopted as a model organism for study by Sydney Brenner in the 1960s when he started a new research program in developmental and neurobiology [1]. During that time, it was also determined that the best food to culture *C. elegans* was *Escherichia coli* (*E. coli*).

Although the main food source of *C. elegans* is *E. coli*, it is important to research the effect of other types of bacteria on the lifespan and reproduction of *C. elegans* [3]. *Pseudomonas fluorescens* and *Bacillus megaterium* will be tested on two types of the nematode, dpy-11 mutant-type and wild-type, and tested to evaluate if the lifespan will either decrease or increase compared to *E. coli*.

This study is conducted to determine if there is a food source for *C. elegans* that is better at improving the lifespan of it compared to *E. coli* [4]. This will be tested by calculating the number of dead worms divided by the number of total worms per plate which is the dead worm percentage (DWP). *B. megaterium* has been shown to cause defects in the fertility of worms, due to their dependence on weak induction of immune genes regulated by the p38 MAPK pathway [3]. Growth from the egg to L2 larvae stage of *C. elegans* on a *B. megaterium* plate was significantly delayed. However, development from the L2 to L4 larvae stage was relatively normal. Additionally, under nutrient-poor conditions, the pathogenic *P. fluorescens* was able to feed *C. elegans* without causing death to the nematodes [3].

From these results, it can be predicted that both *B. megaterium* and *P. fluorescens* will have a fairly long lifespan in both the mutant dpy-11 and wild-type of *C. elegans*. However, *P. fluorescens* will allow the lifespan of the two types of nematodes to be longer. Additionally, the mutant-type dpy-11 of *C. elegans* will have a much longer lifespan, even double, compared to that of the wild-type.

## 2. Materials

12 small sterile petri dishes	Microwave	Sharpie
P-20 micropipette tips	NGM agar	Lighter
<i>Escherichia coli</i> plate	Alcohol burner	P-20 micropipette
<i>Pseudomonas fluorescens</i> vial	<i>Bacillus megaterium</i> vial	Dpy-11 <i>C. elegans</i> plate
Wild-type <i>C. elegans</i> plate	Worm picker	3 sterile cotton swabs
Hot hands	Dissecting microscope	Luria broth
3 vials for inoculation	Colored lab tape	Nutrient agar plates
Inoculating loop		

### 3. Methods

The independent variable of this experiment is the two different pathogens as food source, *P. fluorescens* and *B. megaterium*, for *C. elegans*. The dependent variable of this experiment is the lifespan of the *C. elegans* nematode, which is measured in a percentage of dead worms on each plate. There were four replicates being that there were four plates for every type of pathogen. The controlled variables in this experiment were the temperatures of the incubators, the number of bacteria on each plate, the initial number of worms on each plate, and the recording time.

12 petri dishes with graphs of A - D and 1 - 4 were obtained. Each plate was labeled with "RR, 5B, Bacteria type, *C. elegans* type". The replicate number is four per variable. The label would vary—there were four plates for each type of bacteria, and two of those would be labeled "*C. elegans* wild-type" and the other two would be labeled "*C. elegans* dpy-11." A bottle of Nematode Growth Medium (NGM) agar was loosely capped and put into the microwave, and it was heated on four 30 second intervals until the agar was smooth. Using hot hands and a hot pad, the melted agar was poured into four plates, filled about halfway, and the top was placed back on. Plates settled for 15 minutes to slightly harden and were then placed in the fridge to fully cool until use. An *E. coli* plate, the control group of the experiment, was obtained from the lab supplies. Using a sterile cotton swab, one colony of bacteria was collected and swirled for 30 seconds into a Luria Broth vial labeled "RR 5B *E. coli*."

Using a sterile inoculating loop, the *P. fluorescens* vial was stroked three times to obtain an adequate amount of bacteria. Then, the inoculating loop with the bacteria was swiped on a nutrient agar plate using Z-technique. The plate was put into the bacteria incubator. This step was repeated for the *B. megaterium* as well.

*E. coli* labeled plates with only NGM agar were obtained. Using a P-20 micropipette and sterile tip, 5  $\mu$ L of *E. coli* Luria Broth was drawn up. In the A1 space on the petri dish, the broth in the micropipette was released into the space, and this was repeated for all squares in the grid from A - D and 1 - 4, creating a total of 16 squares. These steps were repeated for all 4 plates of *E. coli*, and the plates were taped on the side with the number plate it was. The number plate and type of *C. elegans* was recorded, and they were placed into the incubator facing up because the liquid would move if they were flipped.

A *C. elegans* wild-type plate and *C. elegans* mutant dpy-11 plate were obtained, as well as a worm picker, ethanol, lighter, and dissecting microscope. The worm picker was dipped in ethanol and lit on fire until the wire turned red. Looking under the microscope, the lid of the *C. elegans* wild-type plate was removed. Two large adult worms were found on the plate. The worm picker was dipped in *E. coli* from the previously made plates. Using the stick bacteria on the worm picker, one worm was picked up, attached to the bacteria, and it was placed in the center of the prepared plate. This would be repeated so that there

were two worms in the middle of each plate. The four plates were placed back into the incubator to let the worms reproduce.

To count the number of dead and alive worms, a plate was placed under the lens of the dissecting microscope. In each individual square, the number of dead worms and number of alive worms were written down. After counting all squares on a plate, the number of dead worms in every square and the number of alive worms in every square were added together and recorded in the lab notebook. The data will be presented as a percentage of dead worms and t-test will be used. The past three paragraphs were repeated for both *P. fluorescens* and *B. megaterium*, *P. fluorescens* being directly after *E. coli* and *B. megaterium* being last.

#### 4. Results

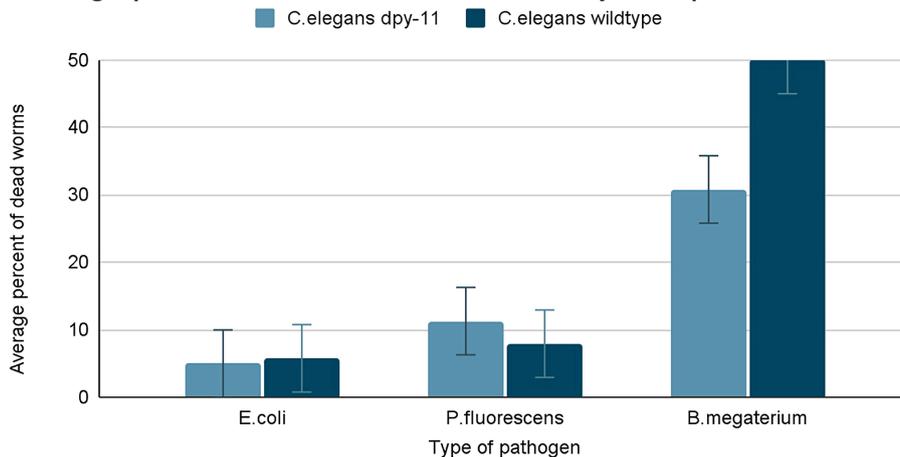
The dead worm percentage of *E. coli* was the lowest of the three pathogens, averaging at about 4.97% for *C. elegans* dpy-11 and 5.75% for the wild-type (Table 1 and Figure 1). This was the lowest percentage of the three pathogens. This was expected as the *C. elegans* dpy-11 grown on *E. coli* was the negative control and *C. elegans* wild-type grown on *E. coli* was the positive control. *P. fluorescens* had a lower average worm death rate compared to *B. megaterium*, meaning it could also be a decent food source for *C. elegans*. From Figure 2, it can be deduced that *C. elegans* wild-type had a higher mortality rate compared to *C. elegans* dpy-11. However, in *P. fluorescens*, the dpy-11 mutant had a higher mortality rate than the wild-type.

Figures 2-4 display the number of dead *C. elegans* over the 4 day recording

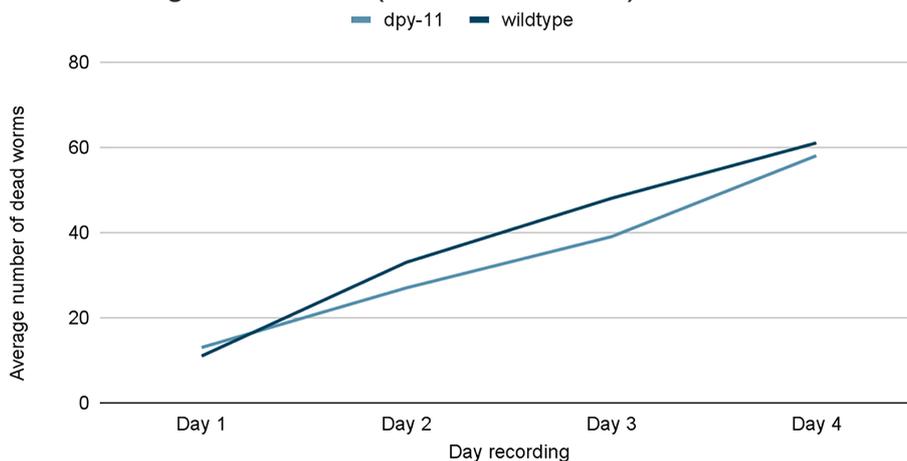
**Table 1.** Percent of dead worms (number of dead worms/number of total worms) over the course of the 4 day counting period each of the pathogens went through.

Type of Bacteria	Day 1 dead worms percentage	Day 2 dead worms percentage	Day 3 dead worms percentage	Day 4 dead worms percentage
<i>E. coli</i> (dpy-11)	2.69%	3.99%	4.94%	8.70%
<i>E. coli</i> (wild-type)	2.46%	4.19%	8.83%	5.74%
<i>E. coli</i> (wild-type)	6.20%	3.42%	6.75%	8.38%
<i>E. coli</i> (dpy-11)	2.92%	2.78%	5.56%	8.19%
<i>P. fluorescens</i> (dpy-11)	12.16%	7.75%	5.85%	19.36%
<i>P. fluorescens</i> (dpy-11)	100%	No worms	No worms	No worms
<i>P. fluorescens</i> (wild-type)	4.49%	1.94%	9.66%	13.87%
<i>P. fluorescens</i> (wild-type)	3.30%	7.39%	9.25%	13.47%
<i>B. megaterium</i> (dpy-11)	31.49%	24.97%	28.11%	0.00%
<i>B. megaterium</i> (dpy-11)	26.46%	31.35%	70.50%	33.80%
<i>B. megaterium</i> (wild-type)	50%	100%	No worms	No worms
<i>B. megaterium</i> (wild-type)	100%	No worms	No worms	No worms

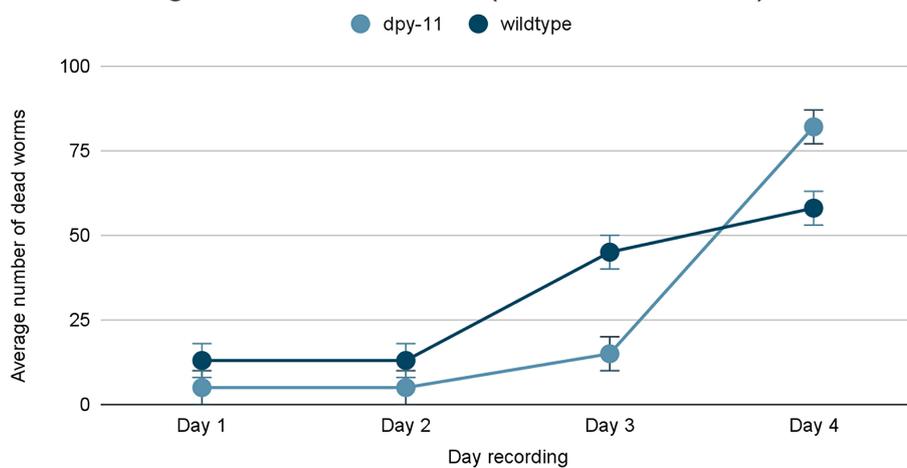
## Average percent of dead worms over 4 day time period



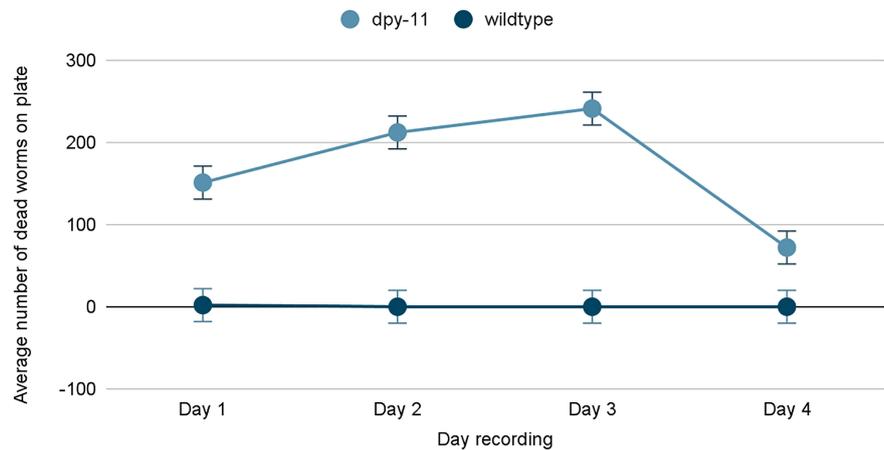
**Figure 1.** Graph representing the average dead worm percentage for each of the pathogens and each type of *C. elegans* mutant.

Dead *C. elegans* on *E. coli* (11/29/21-12/7/21)

**Figure 2.** Average number of dead worms over a 4-day recording time period for *E. coli*.

Dead *C. elegans* on *P. fluorescens* (12/13/21-12/21/21)

**Figure 3.** Average number of dead worms over a 4-day recording time period for *P. fluorescens*.

Dead *C.elegans* on *B.megaterium* (1/6/22-1/14/22)

**Figure 4.** Average number of dead worms over a 4-day recording time period for *B. megaterium*.

period. **Figure 2** displays the average number (from all 4 plates) of dead *C. elegans* on the *E. coli* plates over the 4 recording days. As seen, the number of dead wild-type nematodes grown on *E. coli* were slightly higher than dpy-11 but not by much. As *E. coli* was the control group, the number of nematodes, dead and alive, was a close amount.

In **Figure 3**, the average number (from all 4 plates) of dead *C. elegans* for 4 days on the *P. fluorescens* plates is shown. During the first 2 days, the wild-type had more deaths by a slight amount and by day 3 the wild-type had triple the number of dead worms at 45 while the dpy-11 plates had 15 dead worms. By day 4, the dpy-11 mutant had 82 dead worms while the wild-type had 58 dead worms, directly contrasting the previously stated trends. The varying high number of dead worms may be because the plates had different numbers of total worms. If there was a higher number of total worms, the number of dead worms would most likely be higher.

**Figure 4** shows the average number (from all 4 plates) of dead *C. elegans* for 4 days on the *B. megaterium* plates. The wild-type plates experienced no nematode replication on the plate, and the two worms placed in the beginning died immediately. In this figure, dpy-11 had a large nematode death number, much higher than *E. coli* and *P. fluorescens*. *C. elegans* wild-type did not function on *B. megaterium*.

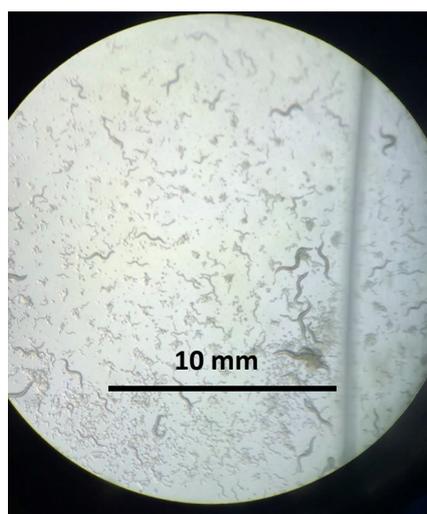
**Table 2** shows t-tests comparing *E. coli* dead worm percentage to independent variables' dead worm percentages. In the figure, it is shown that there is no significant difference between *E. coli* dpy-11 and *P. fluorescens* dpy-11, but there is a significant difference between *E. coli* dpy-11 and *B. megaterium* dpy-11. This is because the *B. megaterium* death rate is much higher than the control group, *E. coli*. There is no significant difference between the wild-type of *E. coli* and the wild-type of both pathogens, *P. fluorescens* and *B. megaterium*. Both bacteria have a death rate in the wild-type that is similar to the negative control.

**Table 2.** t-test comparing *E. coli* dead worm percentage to independent variables dead worm percentages.

	<i>E. coli</i> dpy-11 v. <i>P. fluorescens</i> dpy-11	<i>E. coli</i> dpy-11 v. <i>B. megaterium</i> dpy-11
t value	0.2898	0.0021
	<i>E. coli</i> wild-type v. <i>P. fluorescens</i> wild-type	<i>E. coli</i> wild-type v. <i>B. megaterium</i> wild-type
t value	0.2407	0.1381

**Table 3.** t-test comparing dpy-11 and wild-type *C. elegans* of each bacteria.

	<i>E. coli</i> dpy-11 v. <i>E. coli</i> wild-type
t value	0.5537
	<i>P. fluorescens</i> dpy-11 v. <i>P. fluorescens</i> wild-type
t value	0.4105
	<i>B. megaterium</i> dpy-11 v. <i>B. megaterium</i> wild-type
t value	0.9815



**Figure 5.** Picture of *C. elegans* wild-type grown on *P. fluorescens* (12/13/2021).

**Table 3** shows the comparison of each individual pathogen's death rate and its subsequent types of *C. elegans*, dpy-11 and wild-type. All 3 pathogens, including *E. coli*, had no significant difference between their *C. elegans* dpy-11 death rate and *C. elegans* wild-type death rate.

**Figure 5** is a picture of wild-type *C. elegans* grown on *P. fluorescens*. This shows the numerous worms and how most of the nematodes were in their larvae stage. This is just one cross-section of the plate.

## 5. Discussion

Some errors that occurred consisted of both human error and systematic error. First of all, the method of determining the number of dead worms was an ex-

tremely tedious, difficult task. Sometimes, I would get distracted and start aimlessly counting, so there was definitely room for error in counting. This should not have affected the pattern of the data considering the uncertainty was  $\pm 5$  worms and the number of worms was in the hundreds. The systematic error was a procedural error. For *B. megaterium*, I picked the worms onto the plate with bacteria on 12/23, the day before we left for a 2 week break. The worms were on the plate for over fourteen days before even recording day one. This could have definitely affected the amount of worms on the plate, especially the *B. megaterium* wild-type plate which experienced no growth.

During the initial growth phase (2 days, with the exception noted above) before Day 1 was recorded, the nematodes reproduced at different rates. This resulted in different number of *C. elegans* on Day 1 for the different plates, which is why the percentage deaths was considered along with the absolute number of deaths. Despite this difficulty, it is obvious from the data that *C. elegans* had a longer lifespan with *P. fluorescens* than *B. megaterium*.

A question that arises that was not answered in this study is: which bacteria would *C. elegans* prefer? *E. coli* or *P. fluorescens*. Since nematodes have the ability to make complex decision about the quality of their food [2], conducting a study to see which food source is preferred would be interesting. Also, an experiment considering other bacteria that occur in its natural environment, and combinations of bacteria that would be preferred by a colony, would be an interesting study.

## 6. Conclusion

Conclusively, *P. fluorescens* had a lower nematode death rate than *B. megaterium*, showing that it has a longer lifespan. The hypothesis was supported by the data. *P. fluorescens* had lower death rates (Figure 1) and a lower number of dead worms (Figure 3 and Figure 4). However, *E. coli* did have a lower death rate than both of these bacteria. This may be explained by the gram-positivity of the pathogens. *E. coli* and *P. fluorescens* are both gram-negative organisms (a thin peptidoglycan layer with an outer lipid membrane), so that may explain why *P. fluorescens* promoted nematode life span rather than *B. megaterium*. *B. megaterium* is a gram-positive bacteria (a thick peptidoglycan layer with no outer lipid membrane).

There was no significant difference between the dead worm percentage of *E. coli* and *P. fluorescens*, but *B. megaterium* did have a significant difference compared to *E. coli*. This means *B. megaterium* was less effective at keeping the *C. elegans* alive in both the mutant *dpy-11* and wild-type which experienced no growth. The wild-type of *C. elegans* had a consistently higher rate of death in the nematodes compared to that of *C. elegans* mutant *dpy-11* (Figure 1).

In conclusion, the hypothesis was correct, but *E. coli* is still the best bacterial food for *C. elegans* as it had the lowest nematode death percentage and the lowest number of dead worms. Not only did *E. coli* have the lowest number of dead

worms, but it also had the highest number of total worms for both dpy-11 and wild-type. *E. coli* remains to be the best food source for *C. elegans*, but *P. fluorescens* could be considered as an alternate food source.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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