Chemotaxis Assay: Analysis of *C. elegans* and its relationship with various Microbial Agents

Alex Wen

Introduction

Nematodes such as *Caenorhabditis elegans* has shown to be an excellent model organism for different types of studies, especially host-microbe interaction which is typically based on the interaction between the worms and bacteria. This applies in particular since *C. elegans* can be found in multiple bacterial rich environments, which allows for the possibility to analyze the interaction between both pathogenic and non-pathogenic bacteria (Khan et al. 2018). The nature of the nematodes including the hermaphroditic characteristic of their reproduction process makes it relatively easy to maintain, especially in a laboratory setting (Ruszkiewicz et al. 2018). One of the most impressive nature of *C. elegans* is the chemotaxis behavior of which they use to find food, amongst other things (Margie et al. 2013). Physiologically, *C. elegans* do not have structural eyes or nose for them to attribute sustenance in order to maintain their survival. As such, when dealing with bacteria and analyzing the worms' reaction to different potential food sources, chemotaxis assay is applied to test the nematodes' response for a particular attractant that identifies with their preference in terms of microbial food source.

Hypothesis: The OP50 strain is an auxotrophic strain of the E. coli bacteria whose growth is primarily cultured on NGM agar, in which the material promotes the growth of *C. elegans*. As such, the nematodes should exhibit a higher preference towards the E. coli OP50 bacterial colony.

Experimental Design

I. Aseptic Technique: One of the most prominent factors that needed to be taken account for was that only the bacteria that were being tested were allowed to grow – any presence of other microbial or fungal growth in either the containment plate or the test plate can impact the test results. To mitigate the entrance of outside microbial agents, the lab table was wiped with ethanol before and after any sort of test is conducted upon it. Safety precautions, including goggles and gloves, were ensured. When conducting method development by either passaging the worms or experimentation, a Bunsen burner was lit and the spatula or the pick was thoroughly sterilized by placing them in the flame. Since micropipette tips could not be sterilized with the flame, all tips that were prepared to be used were autoclaved. All agar plates were thoroughly closed unless they are being used. While a plate was being used, it was placed in close proximity to the Bunsen burner such that the convection of the flame diminishes the number of microbial agents in the air from entering an open plate. During the test, the necks of the bacteria containers were heated in

the flame just before pipetting to inhibit the introduction of other bacteria in the air into the samples that were being taken. When not in use, the bacteria are secured in the fridge of 4° C and the worms in 22° C room. Any material wastes that potentially had contact with the worms or bacteria were disposed of in a biological hazard bag.

II. Passaging the Worms: The *C. elegans* needed to be passaged upon a fresh plate with NGM (nematode growth media) and bacteria as food source to ensure their continued survival. As such, 100 µL of the stock E. coli was pipetted onto a fresh NGM plate. After at least 24 hours, a sterilized spatula was used to cut a chunk out of the pre-existent plate with the worms, which was then subsequently transferred onto the newly prepared plate.

III. Bacterial Differentiation: For either a control test or an experimental test, the test plate must be partitioned such that data collection can be as objective as possible. As such, a TSA plate was divided into four quadrants. A 0.8 cm radius circle or square was indicated from the center of the plate as to where the chunked-out worms were placed. Four dots were placed 90° apart and 45° from the boundaries of the quadrant, at 3 cm from the center of the plate – each dot indicates where the different bacteria were placed as the conditions for the experiment. For the experimental tests, each quadrant was labeled with the name of the bacteria being used in the test. When testing for the control, 10 µL of E. coli OP50 was placed into each quadrant at the indicated area. When testing experimentally, 10 µL of E. coli OP50, E. coli K12, M. luteus, and B. subtilis were placed into their respective quadrants at the indicated area. Subsequently, a chunk of worms was passaged onto the indicated location at the center of the plate. The test plates were left alone for 1.5 to 2 hours as the nematodes run their course. After the time period, the number of worms were counted in each quadrant, and the data was recorded. The process is repeated for each plate for multiple trials.

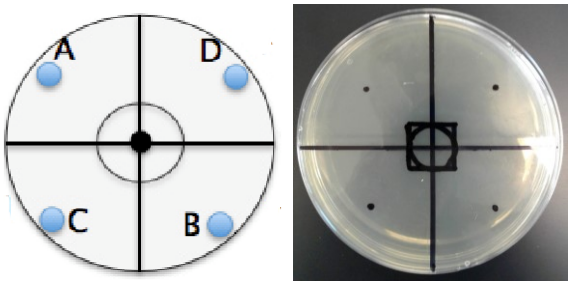


Figure 1 - Schematic of assay

Troubleshooting

I. Method of Worm Transport: The particularity of this chemotaxis assay demands that a large number of worms are placed inside the testing system, in which they will be "sorted" according to their preference. The quantitative nature of this experiment thus requires a method for which the nematodes could be transported efficiently across various plates. Methods that were in consideration were picking, pipetting, or chunking the nematodes to passage them from one plate to another. Picking involves using a sterilized pick coated with bacteria so the worms can stick to it. The picking method is the most effective and accurate way to passage nematodes but they are done in singularities. Since this experiment deals with quantitative analysis, a larger sample size would yield better data for analysis. The pipetting method involves an M9 buffer that would be used to rinse worms off the surface of the agar and pipette them onto a fresh plate. Finally, the chunking method involves using a surface sterilized spatula to cut a cube of agar from the plate with worms onto a fresh plate. Given the time constraints and the amount and type of resources available, the chunking method was used to passage the worms as there was no buffer accessible within a timely fashion to transfer using the pipetting method and the time constraint severely reduces the effectiveness of the picking method.

II. Time: In accordance with establishing the optimal distance and bacteria, another factor that must be accounted for is the amount of time for trial to take. The amount of time for plates to set adds up to at least 24 hours, and the amount of time for the test to run and data collection to take place take roughly an additional 1-2 hours. This does not include the preparation time in terms of the number of plates to setup along with the process of passaging the worms and keeping them alive. As such, given the limited time exposure of this experiment, the tests would best be conducted in a simple environment where the food source and environment are easily accessible to the worms with little to no outside influence that could obstruct or impact the worms' travel path.

    a. Distance: The distance allocation through methods and development refers to the distance between where the bacteria test samples were placed and where the worms were placed. The determination of the optimal distance between the bacteria and

worms is desired to eliminate as much as possible the potential degradation of time's effect on the viability of the experiment.

b. Size of Bacteria: The quantity of bacteria for testing is set such that collection of data is easily ascertainable; as such, each colony of bacteria must be sufficiently large enough such that they are detectable to the worms at an established distance in a reasonable timeframe.

Results

Figure 1 indicate the results of control testing, where E. coli OP50 strain was used to test the effectiveness of the experimentation. The graph established after the test shows a trend, which seems to indicate that the worms were roughly evenly dispersed across the plate between the four quadrants.

Figure 1a – Control Test between groups of E. coli OP50 Strain				
Group 1	*Group 2*	*Group 3*	*Group 4*	# Nematode
32	32	34	28	126

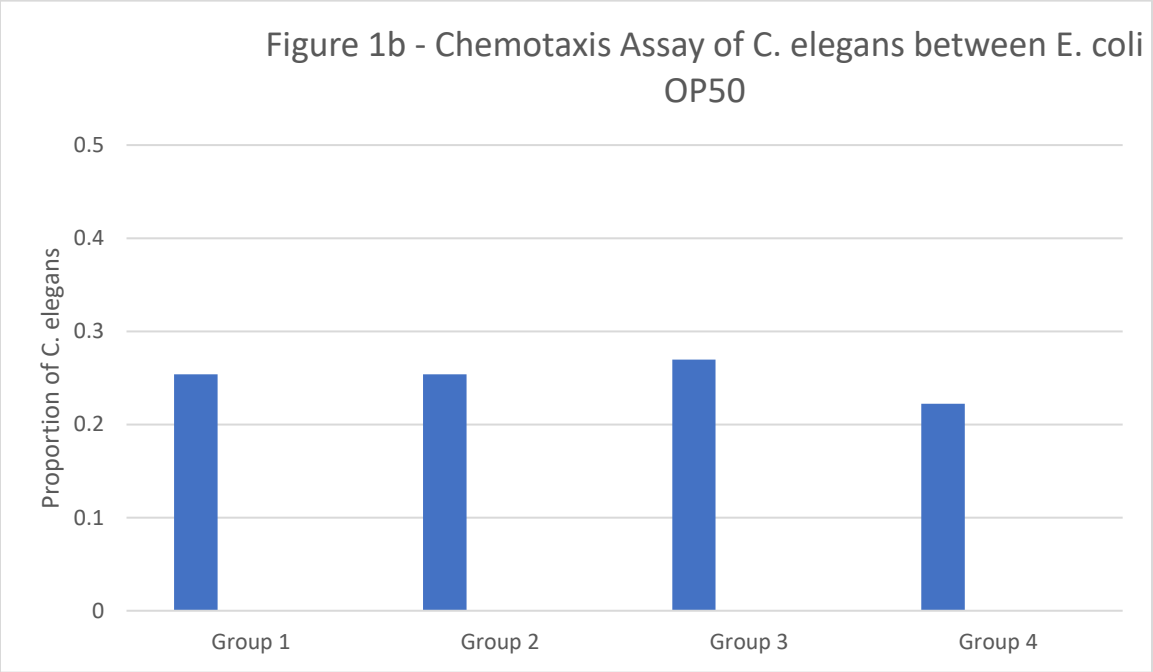
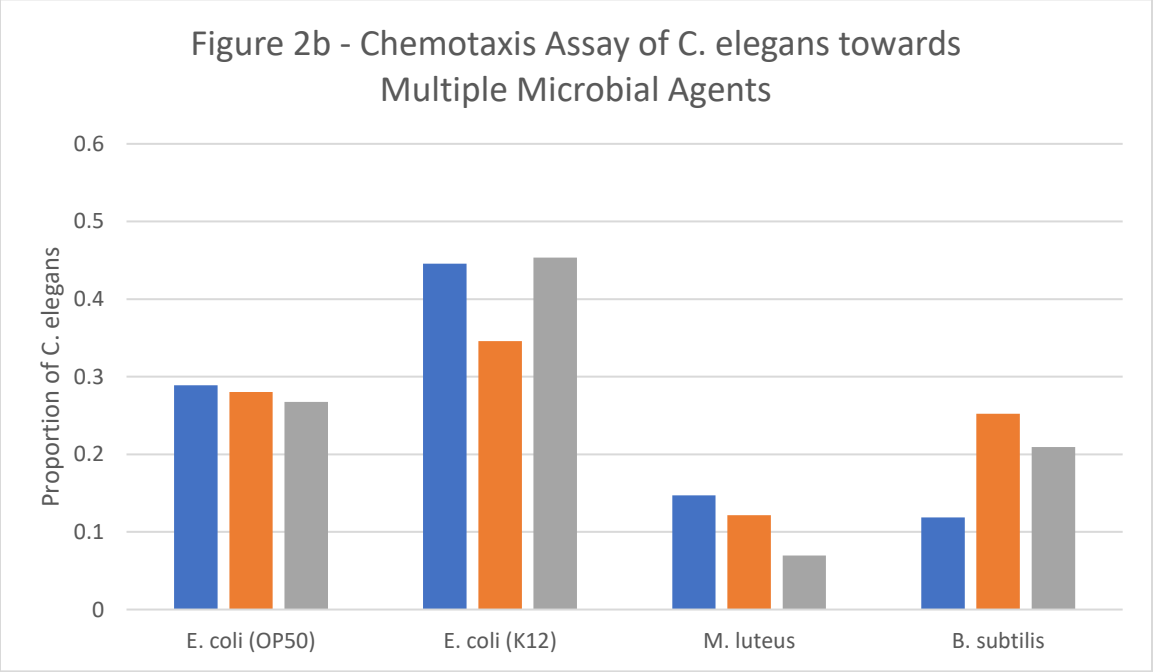


Figure 2 establishes the data collection from the experiment between an average of 3 trials of testing. The graph shows a trend in how worms respond to the different cultures of bacteria placed on the plates. In general, results indicate that worms are attracted to the E. coli K12 strain the most and the M.

luteus the least, with E. coli OP50 strain coming in second and B. subtilis third. In this case, there seems to be a clear preference of microbial food source for the nematodes in general.

Figure 2a – Experimental Test between multiple microbial samples					
TRIAL	*E. coli (OP50)*	*E. coli (K12)*	*M. luteus*	*B. subtilis*	# Nematodes
1	61	94	31	25	211
2	30	37	13	27	107
3	23	39	6	18	86



Conclusion

Since the grouping of nematodes are in accordance with the worms' preference to certain bacterial food source, the presence of the greatest number of worms indicate a higher liking to the particular bacteria. The experimental design shows that the *C. elegans* have a higher partiality to the K12 strain of the E. coli group of bacteria rather than the OP50 strain; as such, the hypothesis is summarily rejected.

Given the limited resources and time allotted for this nature of project, this experiment could be improved upon. One of the potential deviations that could have occurred is due to counting. When

collecting data, individual worms must be differentiated between one and another due to the transparent nature of their physiology established from a bird's eye view. Also, the movement of the worms makes it more difficult to establish an accurate number as part of the data collection. As such, higher accuracy can be obtained if the worms were individually removed from the plate while counting or an anesthetic or other paralytic agent to halt the movement of the worms is applied. Another potential source of error is the presence of any contamination particularly from other bacteria or fungi. The presence of other microbial agents eliminates the viability of the test since their effect is unknown and can subtly influence the trial run – they can even potentially be toxic to the worms, especially if aseptic technique is not thoroughly observed.

Pertinent Questions

1. C. elegans have different forms at different stages of their life cycle, which can potentially include different preference to the microbial sustenance. If worms in each stage of their life cycle are isolated, does this have an impact on the results if each stage is tested?
2. The data collection is analyzed according to the positive results of the experiment (ie. more worms are more partial to one group than the other). Can an inference be made about the negative side of the experiment? In other words, can the worms' aversion to a particular group of bacteria yield productive analyses for real world application?
3. The 2 groups of bacteria that ended with a higher density of worms (E. coli K12 and E. coli OP50) are gram-negative while the other 2 groups that ended with low density (M. luteus and B. subtilis) are gram-positive. Does the gram stain of the bacteria have a particular influence upon the preference of the worms? Can this determination be expanded to make general inferences about the effect of gram stain of a bacteria upon the worms' food source?
4. Since experimental result indicates a preference to bacteria different than predicted, could this potentially influence the interaction nematodes have with plants?
5. This experiment was performed on the basis of the interaction between two groups – worms and bacteria. The bacteria were relatively isolated, but the worms were placed on test plate as a group. Is there any potential effect the interaction between the worms on their chemotaxis behavior?

Works Cited

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