Effects of Green Tea Extract on the Expression of DAF-16 gene in Caenorhabditis elegans
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Background

Caenorhabditis elegans are microscopic, transparent roundworms that are found in temperate environment soils. These microscopic organisms contain many genes, but in this experiment, DAF-16 gene is our focus. Expression of DAF-16 in the intestines of Caenorhabditis elegans seems to increase longevity and stress resistance in these worms (Warnhoff et al. 2014). DAF-16 gene is an ortholog of FOXO-1 gene in Caenorhabditis elegans.

Methods

- Chose an organism of study (C.elegans). Picked a gene (DAF-16) and treatment (green tea extract). Designed primers using “Primer3Plus”.
- Cultured bacteria (OP50 Es.coli) for feeding Caenorhabditis elegans. Placed the worms in the cultured bacteria and incubated in 23°C. Divided the C.elegans into 2 tubes: experimental and control.
- Treated the experimental group with 0.24 g/ml of green tea. Isolated RNAs from the C.elegans with 0.24g/ml concentration of brewed green tea. RNA from C.elegans is alternatively spliced to produce multiple isoforms with different functions.
- Treated the experimental group with 0.24 g/ml of green tea, RNA from C.elegans is alternatively spliced to produce multiple isoforms with different functions.
- Set up PCR for experiment and control cDNA and compared protein isoforms using SMART domain. Ran agarose gel for experimental and control

Hypothesis

When Caenorhabditis elegans are treated with 0.24 g/ml of green tea, RNA from C.elegans gene would be alternatively spliced resulting in different isoforms with different functions.

Figure 1. Map drawing of all the fourteen exons found in REFSEQ. This map is a depiction of DAF-16 gene exon lengths. The black boxes represent exons, and the numbers are the length of each exon measured in base pairs (bp). Box size roughly corresponds to exon size. Lines connecting the exons roughly represent the number of base pairs (introns) between exons. This figure is made with information from Fuchshoehl et al. (2019).

Results

- In figure 3. (b), The experimental product travelled a longer distance than the control.
- PCR product of 300bp band size is seen in figures 3 (a&b) which is close to our expected PCR size. 600bp product is also observed in both figure 3 PCR products, but 600bp product is not what we expected. This could mean that introns that increased the product size were included in the sequence.
- Multiple Reference sequence structures with similar E values are generated when the reference sequence is copied to SMART domain, but there were no result for the isoform (Figure 5).

Conclusion

- The best annealing temperature was 53.9°C because it produced the most visible agarose gel product.
- The control nematodes produced a functional protein, but the nematodes that were exposed to green tea extract did not form the predicted isoforms rather, they produced other isoforms that seemed to be nonfunctional.
- According to a previous study, “lifespan extension [in C.elegans] requires DAF-16, a forkhead/winged-helix transcription factor,” and it does so by blocking and channeling some pathways that affect lifespan (Lin et al. 2001). Our experiment suggests a change in function which could suggest this finding to be true, but further study is needed to prove the validity of this conclusion.
- Finally, this research enabled us to test the prediction we made at the beginning. It may be helpful to this field of study by opening up fields for questions, thereby furthering evaluations and critiques about the validity of our findings.

Future Directions

- Further research could be done to prove if green tea extract would result in increased lifespan among nematodes.
- If someone else would continue this experiment they would need to do PCR again for precision purposes.
- They would also need to be careful on which exons they would go with since DAF-16 is a slightly complicated gene with multiple exon sets.
- Comparing the results obtained from different sets of the available exons when designing primers would also be a task that may be considered.

Literature Cited