Construction of Dietary Reporter Transgenes and the Impact of the Gut Microbiome on Key Metabolic Processes in *C. elegans*

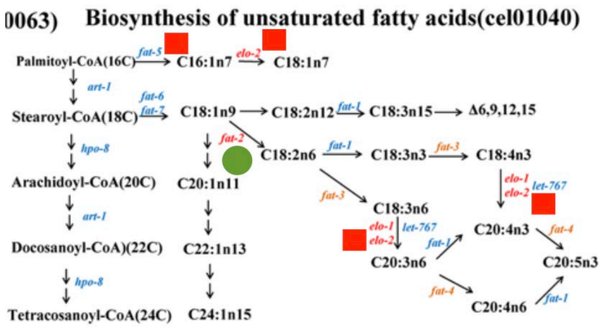
[1] UNC-CH School of Medicine – Department of Cell Biology and Physiology, Chapel Hill, NC, USA
[2]UNC-CH College of Arts and Sciences – Department of Biology, Chapel Hill, NC, USA

Malvika V. Choudhari[1,2],Rachel N. Dumez[1,2], and Robert H. Dowen[1,2]

Abstract

The human microbiome plays a major role in regulation of lipid metabolic pathways and the alteration of its composition through diet is associated with an increased risk of developing metabolic syndromes, such as obesity and diabetes. The interaction between gut bacteria and the nematode, *C. elegans*, can be used to investigate conserved signal transduction pathways that mediate host-microbiome interactions in humans. This project focused on the construction of dietary reporter transgenes and how host dietary pathways respond to microbial signals, including metabolites, to alter *C. elegans* development and aging. Using dietary reporter transgenes, this project will investigate how specific microbes shape key metabolic processes in the worm and through what pathways Kombucha, a fermented tea and purported probiotic, regulates metabolic gene expression and longevity in the animal.

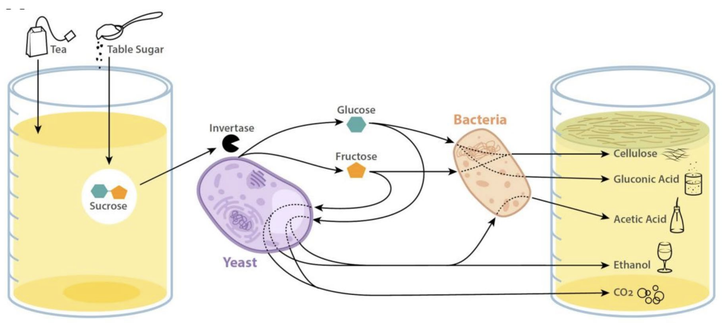
Background



Adopted from Zhang Y, et al. (2013).Comparative genomics and functional study of lipid metabolic genes in Caenorhabditis elegans. *BMC Genomics*. 14(1):164.

C. elegans is a robust, genetically tractable, model system for investigating metabolic regulatory pathways under different environmental conditions. The impact of the gut microbiome on *C. elegans* development and lipid metabolism pathways can be monitored by observing gene expression changes in response to different diets using mRNA-Seq or specific reporter transgenes.

In *C. elegans*, unsaturated fatty acid synthesis is carried out by the fat genes: *fat-5*, *fat-6*, and *fat-7*. All three genes are expressed in the intestine and are orthologs of human SCD-5 (stearoyl-CoA desaturase 5). The expression patterns of these genes in *C. elegans* can be monitored by fusing the promoter sequence of each gene upstream of a fluorescent protein, such as GFP. Once the recombinant vector is successfully injected into the germline, the transparency of *C. elegans* permits the use of fluorescence microscopy to analyze gene expression in living animals.



Adopted from Narayanan M, et al. (2019). Kombucha: a novel model system for cooperation and conflict in a complex multi-species microbial ecosystem. *Peerj*. 7(1):e7565

Due to its associated health benefits, Kombucha has gained immense popularity in recent times. Previous research has suggested the fermented tea enhances cognition, has anti-cancer properties, and extends lifespan. In Kombucha cultures, yeast breaks down sucrose into glucose and fructose and converts it into ethanol. The Dowen Lab has isolated *Acetobacter* from homegrown Kombucha and studies how *C. elegans* metabolizes the acetic acid that is created by *Acetobacter* via conversion from ethanol. Acetic Acid may play an important role in *C. elegans* longevity.

The clec-60 gene, which plays a key role in innate immunity, is strongly induced when Kombucha is fed to C. elegans. We monitor the expression of this gene using a P*clec-60::GFP* transgene (*clec-60* promoter fused to GFP).

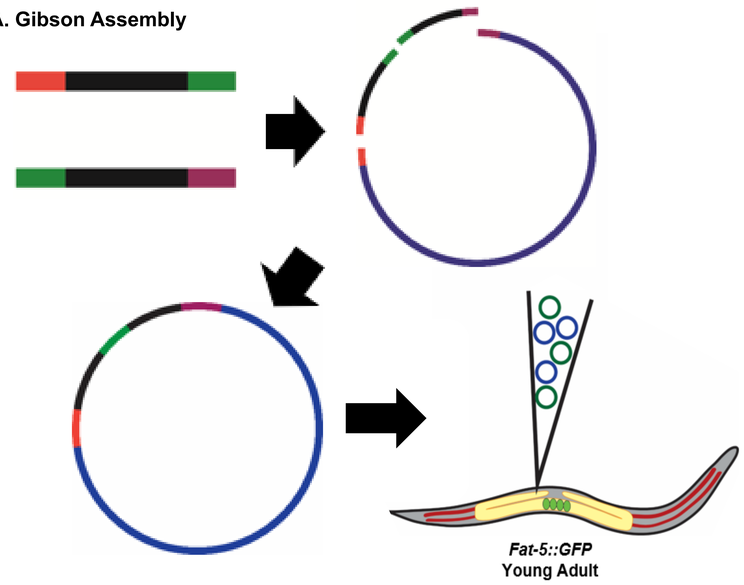
The P*Clec-60::GFP* transgene serves as a readout to compare how *C. elegans* looks on different food sources versus Kombucha. Adding more *Acetobacter* and *Yeast* may elicit higher expression of the transgene and lead to a "simple culture" that supports and influences host physiology & metabolism as detected on Kombucha.

Objectives

- ❑ Construct *fat-5* and *fat-7* reporter transgenes using Gibson assembly. When introduced into *C. elegans*, these transgenes will be used to understand how the microbiome influences essential lipid-metabolism pathways, organismal lifespan, and development in the nematode.
- ❑ Understand what microbes in Kombucha are responsible for the observed physiological phenotypes (i.e. faster growth, smaller body size, and increased longevity) in *C. elegans.*
- ❑ Use P*clec-60::GFP* to find a synthetic mixture of microbes that recapitulates the expression of the reporter when animals are grown on Kombucha culture.

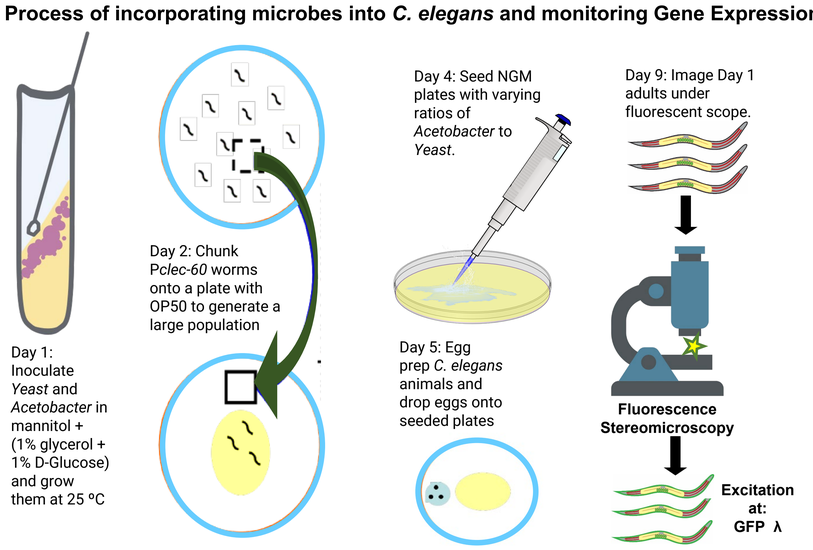
Methods

A. Gibson Assembly



Adopted from Park, A. (2020). MEKK-3 regulates growth and lipid homeostasis in *C. elegans*

B. Process of incorporating microbes into *C. elegans* and monitoring Gene Expression



Results

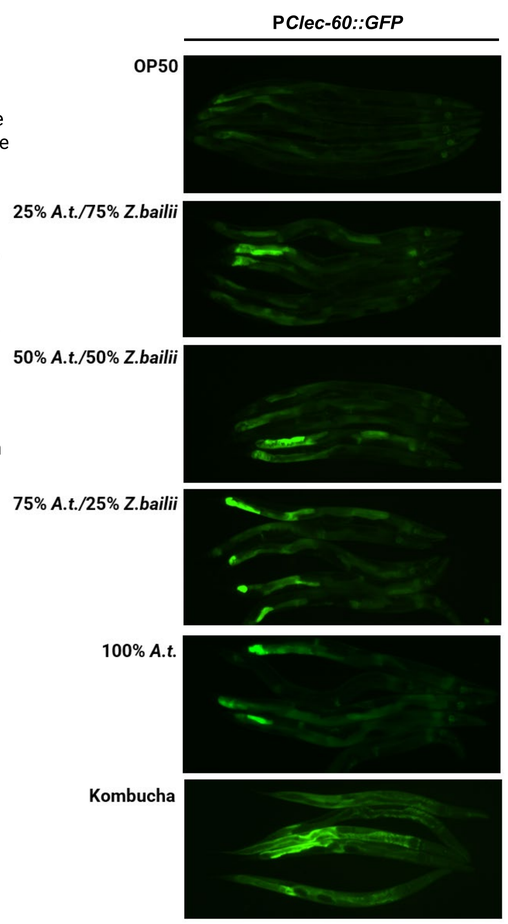


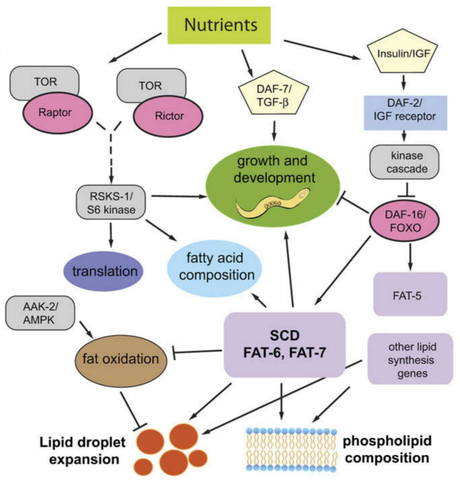
Figure 1. The addition of *Yeast, Zygosaccharomyces bailii (Z.b.),* to *acetobacter tropicalis (A.t.)* leads to increased expression of the reporter as evidenced by the increased brightness in the *C. elegans* intestine. 75 % *A.t*/25% *Z. bailii* is an optimal ratio that almost recapitulates P*clec-60::GFP* expression as observed on Kombucha. Addition of ethanol or another microbe in combination with this ratio may lead to the creation of a "simple culture" that influences C. elegans physiology as seen on Kombucha culture.

Conclusions

- ❑ I successfully generated *fat-5* and *fat-7* transcriptional reporters using Gibson assembly. These transgenes will be injected into C. elegans germlines to generate lipid metabolism reporter strains.
- ❑ The *fat-5* and *fat-7* reporters will allow us to screen these reporters along with other constructs, such as the P*acdh-1::GFP* transgene (involved in short-chain faty acid metabolism), to investigate how *C. elegans* diverts its resources to maintain lipid homeostasis with various diets.
- ❑ Isolation of Acetobacter leads to a phenotype that is similar to the one seen in kombucha.

Future Directions

- ❑ Previous research has shown that downregulating *vit* genes extends the lifespan of C. elegans by stimulating autophagy and lysosomal lipolysis.
- ❑ Investigate how expression changes in *fat-5* and *fat-7* transgenes in response to reduced vitellogenesis.
- ❑ Conduct research with *fat* genes to unravel the complex interactions between the gut microbiome, lipid metabolism, and longevity



Adopted from Shi X, Li J, Zou X, et al. (2013) Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase. *Journal of Lipid Research*. 54(9):2504-2514.

- ❑ Establish *C. elegans'* native microbiome in order to study host-microbiome interactions which were difficult to understand with synthetic communities.
- ❑ Create a simplified culture of microbe(s) that recapitulates the longevity phenotypes observed in populations consuming Kombucha.
- ❑ Sequence Kombucha culture and work to identify which combination of microbes are needed to recapitulate the observed metabolic and longevity phenotypes.
- ❑ Investigate if Kombucha increases longevity in an animal that has a more traditional microbiome.

References

[1] Zhang Y, Zou X, Ding Y, Wang H, Wu X, Liang B. (2013).Comparative genomics and functional study of lipid metabolic genes in Caenorhabditis elegans. *BMC Genomics*. 14(1):164. doi:10.1186/1471-2164-14-164
[2] Aoun, A., Darwish, F., & Hamod, N. (2020). The Influence of the Gut Microbiome on Obesity in Adults and the Role of Probiotics, Prebiotics, and Synbiotics for Weight Loss. Preventive nutrition and food science, 25(2), 113–123. https://doi.org/10.3746/pnf.2020.25.2.113
[3] Boulin, T et al. (2006). Reporter gene fusions. The C. elegans Research Community, WormBook. doi/10.1895/wormbook.1.106.1, Brock, T. J., Browse, J., & Watts, J. L. (2006). Genetic regulation of unsaturated fatty acid composition in C. elegans. PLoS genetics, 2(7), e108. https://doi.org/10.1371/journal.pgen.0020108
[4] Elle, I. C., Rødkær, S. V., Fredens, J., & Færgeman, N. J. (2012). A method for measuring fatty acid oxidation in C. elegans. Worm, 1(1), 26–30. https://doi.org/10.4161/worm.19564
[5] Gibson, D., Young, L., Chuang, RY. et al. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods 6, 343–345. https://doi.org/10.1038/nmeth.131
[6] Seah, N. E., de Magalhaes Filho, C. D., Petrashen, A. P., Henderson, H. R., Laguer, J., Gonzalez, J., Dillin, A., Hansen, M., & Lapierre, L. R. (2016). Autophagy-mediated longevity is modulated by lipoprotein biogenesis. Autophagy, 12(2), 261–272. https://doi.org/10.1080/15548627.2015.1127464
[7] Shi X, Li J, Zou X, et al. (2013) Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase. *Journal of Lipid Research*. 54(9):2504-2514. doi:10.1194/jlr.m039669
[8] Narayanan S, et al. (2019). Kombucha: a novel model system for cooperation and conflict in a complex multi-species microbial ecosystem. *Peerj*. 7:e7565. DOI: 10.7717/peerj.7565.

Acknowledgements

Special thanks to Dr. Robert Dowen for his help in gathering data and mentoring the research project. I would also like to thank Rachel DuMez for her assistance with laboratory techniques as well as her guidance throughout this project.