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The Role of Tryptophanase in bacterial survival in the Gastrointestinal Tract of *Drosophila melanogaster*

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Introduction

- Populations of bacteria in the gastrointestinal tract survive in mixed biofilms formed near sugars and other necessary resources for cellular metabolism.
- The gene *tnaA* is linked to a bacterial colonization method in *E. coli* involving the production and secretion of indole, a signaling molecule linked to the decomposition of biofilms. The production of indole is induced in a high tryptophan low glucose environment.
- A strain of *E. coli* was produced with a mutation in *tnaA* that rendered it inoperable and was introduced into several populations of *Drosophila melanogaster* in order to study the importance of *tnaA* to the bacteria's colonization and survival.

Figure 1.

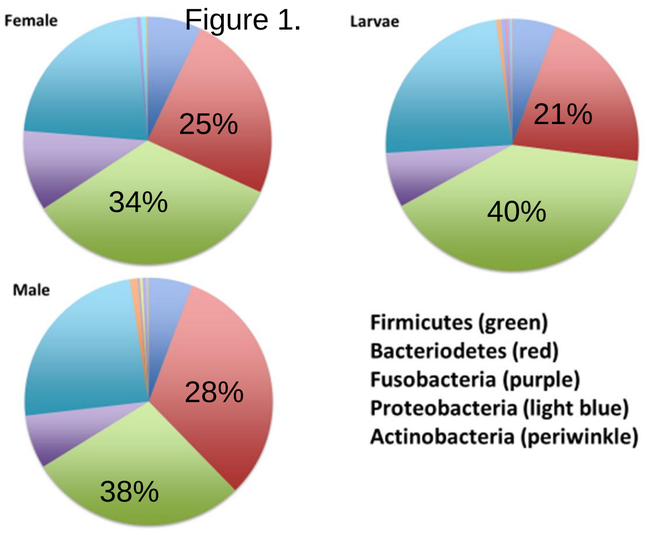


Figure 1. The percentage of Bacteriodetes and the percentage composition of Firmicutes are inversely proportional to one another. *E. coli* was represented in the overall population of bacteria at about 1%.

Experimental Overview

Figure 2.

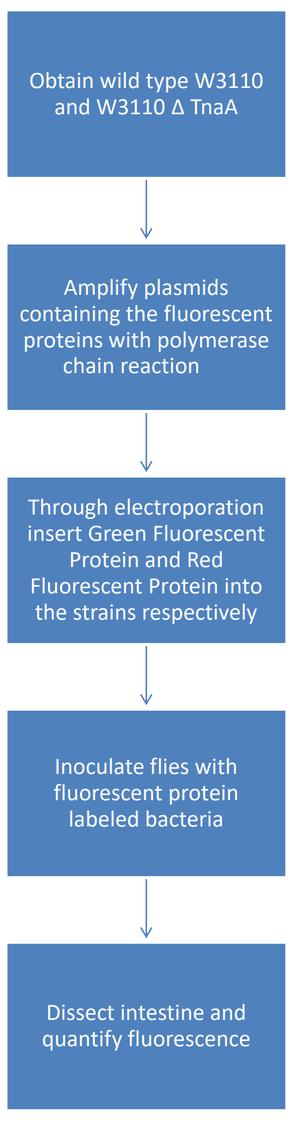


Figure 2. details an outline for the overall experimental goals and procedures in each step of the process

Figure 3

a. b.

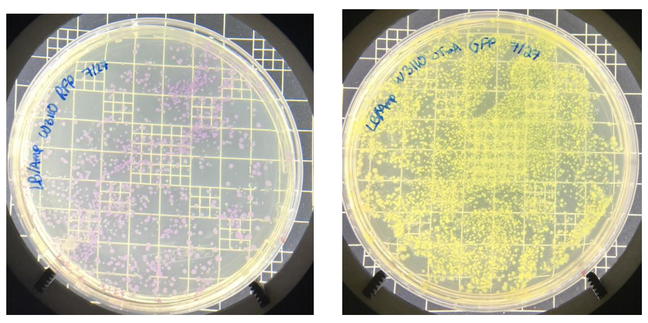
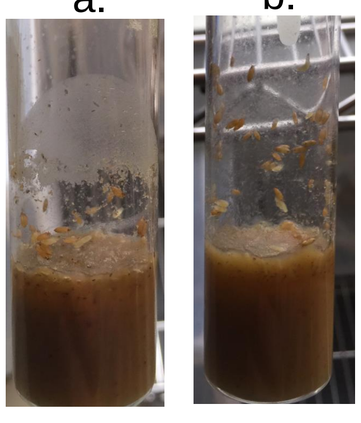


Figure 3 (a) is an isolated population of wild type *E. coli* grown on LB with ampicillin while (b) is a population of Δ*tnaA E. coli*.

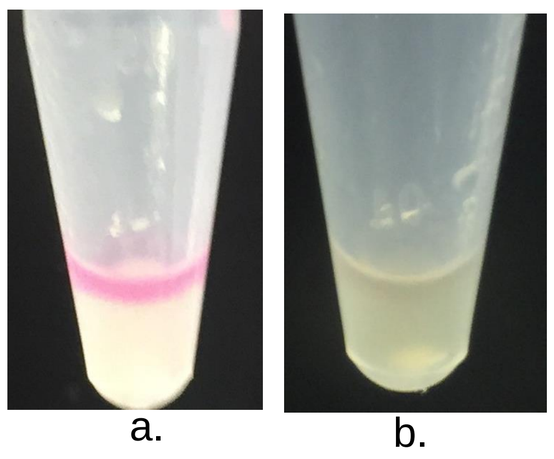
Figure 4.

a. b.



The third instar *D. melanogaster* larva in figure 4 (a) are the first generation raised on antibiotic food and the larva in figure 4.(b) were raised on food without antibiotics

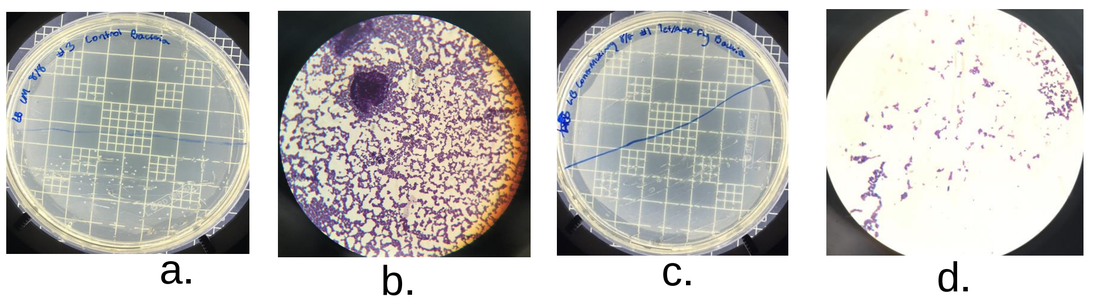
Results



a. b.

Figure 5. Using Kovac's reagent the presence of indole was confirmed in the wild type(a) while the *tnaA* mutant(b) tested negative.

Figure 5.

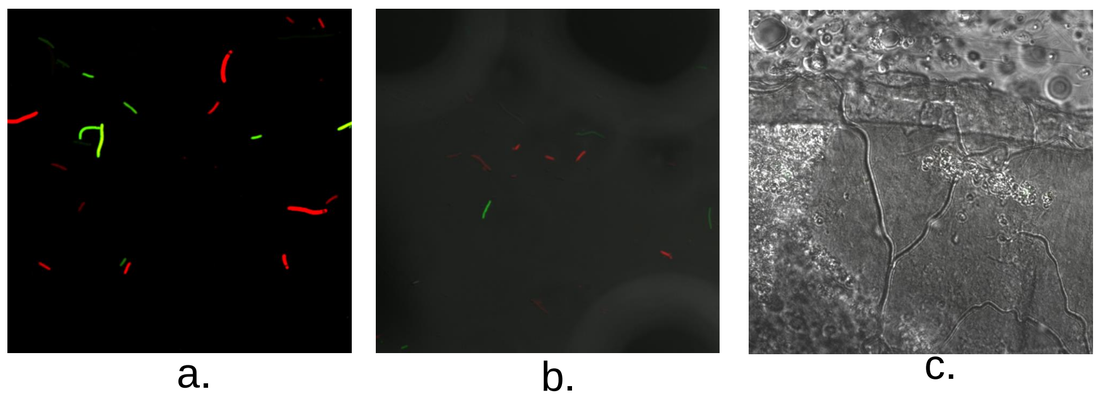


a. b. c. d.

Figure 6.

Figure 6.(b, d) Is a gram stain of the bacteria growing on the plate(a, c). The bacteria is identified as a lactobacillus. The bacteria were grown from the intestine of *D. melanogaster* raised on antibiotics(c, d) and without antibiotics(a, b)

Figure 7.



a. b. c.

Figure 7 (a) is a mixed group of bacteria producing GFP and RFP to quantify fluorescence; while (b.) is a slightly underproduction group of RFP and GFP producing bacteria. The gut in (c.) contains no GFP or RFP producing bacteria.

Once the intestines of the *D. melanogaster* were dissected out, they were placed on a slide and quantified for fluorescence.There was no visual detection of bacterial produced fluorescence from within the intestine. In addition some bacteria appeared to produce less fluorescent protein over time and lose their fluorescent indicator .

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Conclusion

Future Directions

In the future more genes that relate to trypophanase operon can be labelled with fluorescent proteins to better understand their role in contributing to bacterial survival *in vivo.* In addition the same model organism can be inoculated with both mutant and wild type bacteria to better understand competition within species between the two bacterial strains. The recombineering method should be used in the future to directly replace *tnaA* and other key genes with the fluorescent proteins.

Acknowledgements

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