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Effects of Microbiota on Starvation Resistant Drosophila Melanogaster

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Abstract

Bacteria in modern society have often been perceived as having negative effects on humans with complications and potential death to their hosts. In recent years, the gut microbiota has shown that not all bacteria inhabiting a host cause negative side effects, but they instead can provide essential nutrients to their host and even directly impact growth rate and development. In this study, axenic *Drosophila melanogaster* were generated through egg dechorionation with 7% bleach to test the effects of the absence of commensal bacteria on the flies growth and development. Lipid content was recorded of control and axenic fruit flies from six different populations: FA, FB, FC, SA, SB, SC. On average, the axenic flies took approximately three days longer to development compared to the control. Starvation resistant axenic flies that survived had lower lipid content compared to their control counterparts.

Introduction

Microbiota is defined as a particular area that is inhabited by microorganisms such as bacteria, fungi, archaea, and viruses. From an immunology standpoint, bacteria that occupy a host are often perceived as a pathogen. Recent studies have shown that bacteria that inhabit the gut of animals and humans can have positive effects on host nutrition and growth development. Bacteria can provide essential coenzymes such as B-12 or produce enzymes that can digest materials that the host cannot on their own. The interaction of the host and bacteria can be further investigated by studying if the host's genes can affect what type of bacteria that inhabit and influence it. This study investigated whether or not the presence of commensal bacteria in the gut microbiota of starvation resistant *Drosophila melanogaster* impacts the growth and development of these genetically obese flies. For this study, it is hypothesized that the absence of gut microbiota of the starvation resistant flies (S flies) will result in slower development and decreased growth. The S flies do have large lipid content, but without the aid of a gut microbiota the flies lose out on essential coenzymes and nutrients that bacteria provide that contribute to growth. A control with commensal bacteria and an axenic control that is sterile are compared in this study.

Methods

Egg Harvesting

For this experiment there were six different populations utilized: FA, FB, FC, SA, SB, and SC. The F populations are flies that are fed and the S population are the starvation resistant flies. Grape agar plates with yeast were placed onto the bottles of each population. After 20 hours in a 25℃ incubator, eggs were collected from agar plates with a paint brush and distilled water onto a separate mesh for each population. Approximately, 30 eggs were brushed onto labeled bottles of autoclaved cornmeal media for each population. These eggs represent the control flies that have commensal bacteria. A limited amount of eggs were plated onto MRS and Brain Heart agar to test for bacterial presence.

Dechorionating Eggs

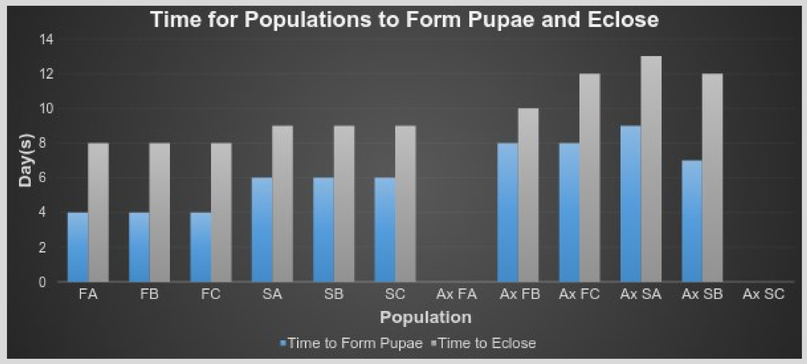
Turn on UV light in tissue culture hood for one hour. After one hour, the materials used for dechorionating eggs are placed into the hood covered in ethanol. The UV light is turned on again for another 15 minutes. A mesh containing a single population of eggs is placed into a bushing.

This bushing facilitates the dechorinating process by bleaching the eggs in 7% bleach for 4 minutes and rinsing off with sterile milli-Q water with three washes. Approximately, 30 eggs were placed into a labeled bottle that contained autoclaved cornmeal media. This was repeated with fresh bleach and water for each respective population. Each population had eggs crushed onto MRS and Brain Heart agar to test for successful bleaching. The control and axenic flies were both incubated at 25℃. The flies were checked everyday to observe formation of pupae and flies.

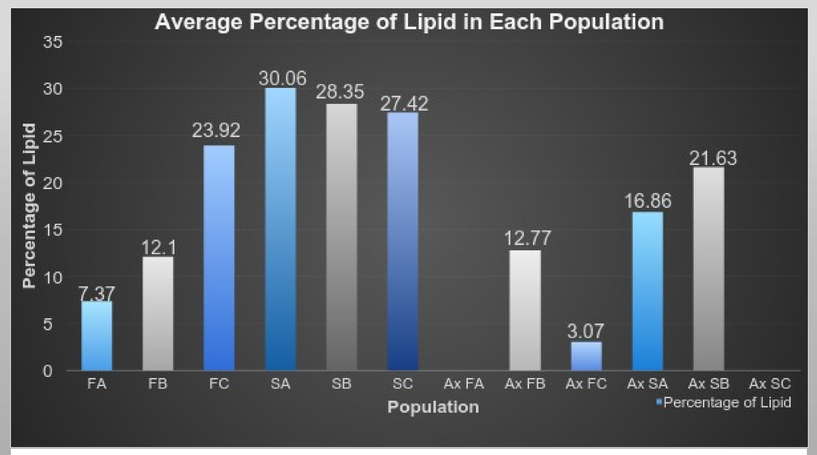
Triglyceride Test

After four days of the flies emerging, the bottles were frozen (this will occur at different times due to differing development times) to kill the flies. Ten flies were placed into microcentrifuge tubes. The tubes were left open to dry in a 50°C oven over night to dry. The dry weight of each individual fly was recorded with a microscale. All of the tubes were filled with hexane and sat over night once more. The hexane was removed and the tubes were dried for an hour in the oven. The flies' dry weight was recorded after drying. The difference between the dry weight and the hexane dry weight is the lipid content of the individual fly.

Results



The observed axenic populations eclosed on average 3 days later compared to the control population. The axenic FA and SC populations did not have any flies eclose.



The average lipid content and percentage was recorded for each population. The axenic FC and SA populations only yield one fly per population, thus a statistical test would result in there being no significance.

Discussion

The axenic populations FA and SC both did not rear any flies. This could have occurred due to the eggs not surviving being in the 7% bleach during the egg dechorionating process. The bleaching step is a possible reason for the rest of the axenic populations to yield low amounts of pupae and flies when compared to their control populations. Despite the low amount of axenic flies reared, there is an observed delay in development of pupae formation and eclosed adult flies for the sterile flies. On average, the axenic flies had a three day delay in becoming adults versus to the flies with commensal bacteria.

A statistical test for significance was not performed for the lipid content since all of the axenic populations did not have ten or more flies. Despite that, the surviving axenic flies are observed to have lower lipid content than the control. The Ax FB population is the exception to that with a 0.67 increased percentage. All of the axenic plates showed no bacterial growth except for the Brain Heart agar for the Ax FB plate. It had three bacterial colonies, but that does not indicate contamination. Fifty colonies or more is an indication of contamination, but that does not fully indicate that this population was not truly contaminated. For future experiments, more eggs should be used for the bleaching process to yield more adult flies. Having more adult flies can allow the ability to plate adult flies to observe if their gut was colonized by bacteria.

Conclusions

Despite not having enough axenic flies to survive the bleaching process, the observed results display that both fed and starved axenic fly populations had delayed growth compared to the flies with commensal bacteria. The axenic starvation resistant fly populations both show that they have lowered lipid content as well. There are no statistical tests for significance for this project due to the lack of axenic flies. For future study, the amount of eggs collected should be increased in order to have more that survive the bleaching process. Without the test for significance to confidently back this claim, these results support the proposed hypothesis that axenic starvation resistant flies will have delayed growth and lower lipid content.

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