Drosophila melanogaster microbiome increased in diversity after exposure to Benzo [a] pyrene in females more than males

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Summary

- We examined how B[a]P affects the microbiota of fruit flies in different sexes
- The 16S sequencing was used to identify and measure the abundance of bacteria
- The female fruit flies microbiota became more diverse after B[a]P exposure

Abstract

B[a]P is made from the combustion of organic matter, such as fuel and cigarettes. It is commonly found in air pollution, and it is known to cause cancer. B[a]P's effect on the microbiota of organisms is not well known. We treated fruit flies (*Drosophila melanogaster*) with 7-day dietary exposure of two B[a]P doses with proper vehicle and negative control. We then extracted total DNA from the flies and sequenced the V4 region of the 16S gene. We found that the female flies' microbiota became more diverse following B[a]P exposure compared to the males. Additionally, we also observed that the abundance of Providencia, a genus of pathogenic bacteria, increased in the vehicle control and decreased in the exposure conditions.

Introduction

Hypothesis: The microbiota of both male and female *D. melanogaster* will be more diverse with the dietary exposure of Benzo [a] pyrene.

Air Pollution

- Negatively affects human brain function, immune system, and cardiovascular system (1)
- Benzo [a] pyrene (B[a]P)
  - A type of polycyclic aromatic hydrocarbon, a group of toxic compounds that can change gut microbiota composition in human children and mice (2,3)
  - Produced from burning organic compounds, such as fossil fuel (4)

***D. melanogaster* (Fruit Fly)**

- Advantages
  - Fast reproduction
  - Easy and inexpensive to maintain
- Sexual dimorphism
  - Easily distinguishable genitalia and color differences on legs and abdomen allow separating the two sexes for exposures
- 60% of fruit fly DNA is identical to human DNA (5)

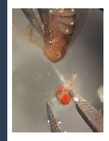
16s Gene and Microbiota

- Microbiota: all the microorganisms in an environment, including bacteria, viruses, and fungi
- 16s gene: present only in bacteria, allows analysis of bacteria within the microbiota instead of all extracted DNA
  - Targeted the variable region, V4, to distinguish species of bacteria

Methodology

Fruit fly sexing: Males and females can be differentiated through different coloring on their abdomen and legs and the presence of genitalia.

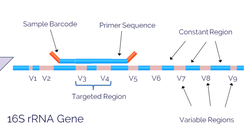
Exposure: Since birth, fruit flies were exposed to polluted food for 7 days (B[a]P was dissolved in acetone, which was then mixed into the food)



Flies dissection: Sanitize flies before dissection in ethanol to remove external bacteria. Fly dissection consists of dismembering their legs, head, and wings.

DNA

DNA extraction with Qiagen Blood & Tissue Kit: Fly bodies were ground with tissue homogenizer pestles. Lysis was performed to extract the DNA. The DNA was bound to the column and washed. Purified DNA was then eluted and quantified with Nanodrop.



Polymerase Chain Reaction:
The V4 region of the 16S gene was amplified

Primers used:
16S V4 Forward
5'GACGCTCTTCCGATCTTATGGTAATTGTGTGCCAGCMGCCGCGGTAA3'
16S V4 Reverse
5'TGTGCTCTTCCGATCTAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT3'

DNA Sequencing: with MinION Nanopore sequencer to obtain reads.

Analyze Sequencing Data: Oxford Nanopore EPI2ME analysis platform was used to match sequencing reads to bacteria species.

Results

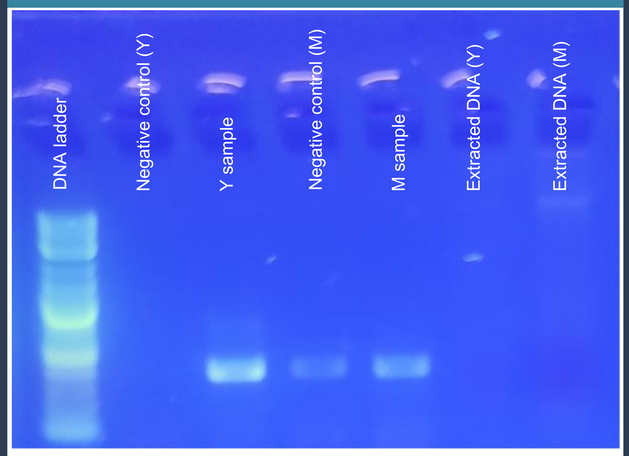


Figure 1. Amplified V4 region of the 16S gene in fruit flies. Agarose Gel Electrophoresis (AGE) was used to visualize the PCR amplification products. 100 ng of extracted DNA was put into the PCR reaction. 150V was applied to the agarose gel (1.5%) for 30 mins. All amplified samples are ~300bp.

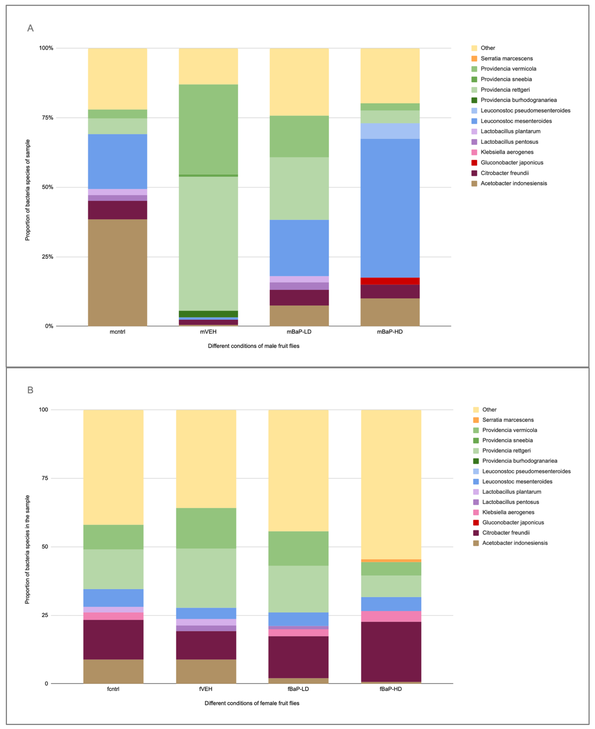


Figure 2. Top seven bacteria species read within the microbiota of fruit flies, differentiated by sex: A) Males, B) Females. Percentages of bacteria abundance in the samples were calculated by dividing the reads of a bacteria by the total number of classified reads in the samples. The first column is the negative control. VEH is the vehicle control with acetone. LD stands for low dose of B[a]P, and HD stands for high dose of B[a]P.

Discussion

Our hypothesis was partially correct: Only the females' microbiota became more diverse after dietary B[a]P exposure.

- **The proportion of reads in the "Other" category, which contains all classified reads besides the top seven species, increased more in females than males.**
  - Since gut microbiota is heavily dependent on the sex of the organism, we predicted that different dosages of B[a]P would produce different results within the male and female microbiota (6).
  - Females exposed to a high dose of B[a]P increased in diversity. The proportion of reads in the "Other" category increased from 35% (VEH) to 44% (LD) to 54% (HD) (Figure 2).
  - In males, the percentages fluctuated from 13% (VEH) to 24% (LD) to 19% (HD).
- **Most abundant bacteria detected are common in fruit flies**
  - Fruit fly microbiota is known to be predominantly composed of bacteria including: Acetobacter, Gluconobacter and Lactobacillus (7).
- **Fluctuation of pathogen abundance under different conditions**
  - Providencia, a prevalent pathogen found within our samples (Figure 2), is a common pathogen among humans and insects, including fruit flies (8).
  - Noticeable increase in pathogens for both sexes under vehicle conditions, especially males (Figure 2).
  - Providencia decreased as B[a]P dosage increased in both sexes.
    - In males, the decrease in Providencia is more pronounced than in females.
- **Unique top seven bacteria in high b[a]p dosages**
  - *Serratia marcescens*, a bacteria that became more relatively abundant in high B[a]P females (Figure 2B), can be fatal to fruit flies if it enters their hemolymph (9).

Limitations/Future Directions

- There was only one replicate for each condition.
  - Further experiments must be conducted to see if our findings are consistent among the replicates.
- The flies that were collected were not consistent in age.
  - Future experiments can be conducted to promote consistency with results and test whether age affects the microbiome.

References