Benzo[a]pyrene decreases the abundance of two pathogens from the genus *Providencia* in *Drosophila melanogaster*

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Abstract

B[a]P is a widespread toxic chemical known to cause cancer and compromise the immune and reproductive systems. B[a]P has also been shown to impact the microbiota of *Chlamys ferrari*, a species of scallop. We hypothesized that the genetic diversity of the microbiota of *Drosophila melanogaster*, fruit flies, would decrease as the amount of B[a]P increases. Total DNA was isolated from flies fed food with B[a]P dissolved in acetone. We sequenced the V4 region of the 16S rRNA gene to identify the species of bacteria in the fruit flies. The most abundant species are common gut bacteria or pathogens in fruit flies. Higher levels of B[a]P decreased the abundance of the pathogens.

Introduction

Hypothesis

In both female and male fruit flies, the microbiota's diversity is lower with higher B[a]P exposure.

Benzo[a]pyrene (B[a]P)

- B[a]P is present in water, air in urban areas (especially with heavy traffic), kitchen stoves, and cooking oils.[1]
  - Health risks: lung cancer[2], impaired memory, and impaired spatial learning [3].
- B[a]P may have a significant impact on health due to changes in microbial composition.
  - A study on *Chlamys farreri* (scallops) showed that exposure to B[a]P changed the composition the gut microbiome.[4]

Microbiome

- Microbiota is correlated to gene expression. [5]
- The lack of microbial diversity in humans is linked to the risk of diseases.[6]

Males vs. Females

- A study showed that B[a]P-exposed female mice showed faster motor learning, while exposed male mice were slower. [7]
- Males have different hormonal compositions compared to females in humans. [8]
- The composition of microbiota differs between *Drosophila Melanogaster* sexes.[9]
- Males and females have different physiological properties.
  - In a study involving physiological responses to submaximal exercise between men and women, men showed higher oxygen uptake, blood pressure, and lactate concentration than women.[10]

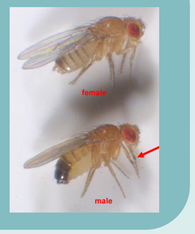
Fruit flies (*Drosophila melanogaster*)

- Sexually dimorphic.[11]
- Cost-efficient to maintain.[12]
- ~60% of fly genes are homologous to humans'. [13]

Methodology

Fly Maintenance and Tissue Collection

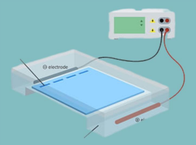
- Exposure condition: standard cornmeal food (control); food with 452.2 mM acetone (vehicle control), 0.2 mM B[a]P (low dose), 1 mM B[a]P (high dose)
- 10 female flies and 10 male flies housed together and allowed to mate
- 10 flies per sex of each condition used for DNA extraction



DNA Isolation

- QIAGEN DNeasy Blood and Tissue Kit was used
  - following manufacturer's protocol

Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis (AGE)



- One reaction prepared with no DNA (negative control), one reaction prepared with DNA from a golden retriever mouth swab (positive control)
- Promega GoTaq Green Master Mix, following the manufacturer's protocol
  - Forward primer: 5-GTG YCA GCM GCC GCG GTA A-3
  - Reverse primer: 5-GGA CTA CNV GGG TWT CTA AT-3
- All PCR products ran with AGE: 1.6% agarose gel, at 105V

16S Sequencing and Data Analysis

- Library prep using the Nanopore 16S Barcoding Kit
- Performed on Oxford Nanopore Sequencer: MinION, with R9.4.1 flow cell
- Analysis done on EPI2ME platform with 16S Classification workflow v5.0.6
- Number of most abundant species covering 45% of classified reads used to determine diversity within samples

Results

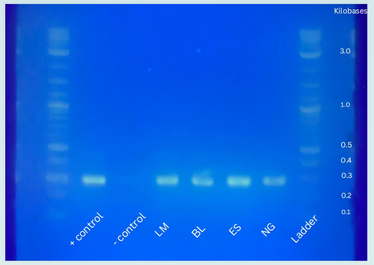


Figure 1. Gel electrophoresis verification of PCR amplification. The left and right columns are DNA ladders. Each band between the markers represents a sample of DNA. The labels on the right indicates the size of the DNA in base pairs.

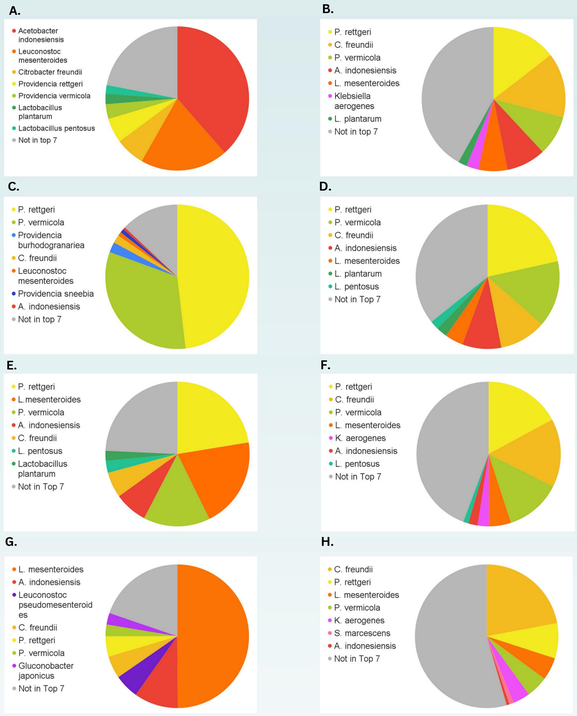


Figure 2. Microbiota species abundance in the negative control (A-B), vehicle control (C-D), low B[a]P (E-F), and high B[a]P (G-H) conditions. Males are shown in A, C, E, and G; females are shown in B, D, F, and H. Percentages were determined by dividing a single species' classified reads by the total number of classified reads.

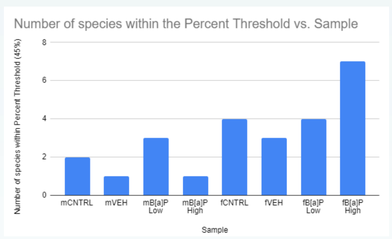


Figure 3. Female microbiota diversity increases with higher B[a]P levels; the relationship is non-linear in males. Microbiota diversity was evaluated with the number of most abundant species covering 45% of classified reads.

Discussion and Conclusion

- Female fruit flies exposed to higher B[a]P levels showed an increase in microbiota diversity; males exposed to high B[a]P levels showed a decrease in microbiota diversity compared to low B[a]P levels (Figure 3).
  - Microbial composition of male mice changed following B[a]P exposure.[14]
- In both males and females, *P. rettgeri* and *P. vermicola,* two pathogens in fruit flies [15], decreased in abundance with increasing B[a]P levels (Figure 2C-H), but the changes in the other bacteria's abundance are drastically different between the two sexes.
  - In males, *L. mesenteroides* has the greatest abundance increase from the vehicle control to high B[a]P exposure, followed by *A. indonesiensis* (Figure 2C,E,G). The drastic increase in these two species abundance kept the overall diversity relatively low.
  - In females, most of the other species increased in abundance with higher B[a]P levels, resulting in higher microbiota diversity (Figure 2D,F,H).
- Compared to the negative control, both males and females exposed to acetone have decreased microbiota diversity.
  - The decreased diversity is mostly characterized by the increased abundance of *P. rettgeri* and *P. vermicola*, and decreased abundance of *A. indonesiensis* and *L. mesenteroides* (Figure 2A-D).
    - *Acetobacter* and *Lactobacillus* are bacteria commonly found in adult fruit flies.[16]
    - The presence of acetone, the vehicle for B[a]P exposure likely disrupted the native microbiota in fruit flies.

Study Limitations and Future Directions

- The age of the flies was mixed, which could affect the data; in the future, flies of the same age should be used
- DNA should be extracted only from the gut, which is more relevant to health
- Nanopore 16S sequencing is a relatively new technology [17], and the results should be verified with more accurate and established sequencing methods
- Experiment should be performed on more replicates

References