Microbial interactions of necrophagous flies and their impact on bacterial transmission over time

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Abstract

Flies frequently interact with humans, as well as bacteria-rich environments. The roles in which flies are involved in bacterial transmission were investigated using next generation (NGS) Illumina Hiseq X 150bp paired-end sequencing and bioinformatic analysis. The microbiomes of flesh flies (*Sarcophaga bullata*) and decomposing rat tissues were compared over periods of time following rat exposure to Sarcophagid flies. Sequencing results were analyzed for presence of targeted antibiotic-resistant bacteria from the 2017 World Health Organization (WHO) Priority List using the Kraken database. A separate database was constructed using 16s sequences from bacteria of interest. Blast+/cd-hit-2d compared this database to our 16s sequences to identify our selected bacteria of interest. Spread plate dilutions were also made to observe relationships between culturable bacteria on each of the samples. Sequence comparisons revealed variance in microbiomes between different time points, as well as between the flies and rat tissue. Eleven of the WHO prioritized antibiotic-resistant pathogens were found on each of the samples. Spread plate dilutions showed larger amounts of cultured bacterial colonies on rat tissues than fly tissues, as well as an average decrease in bacterial concentrations over time. From this study, we concluded that the microbiomes of flies are influenced by exposure to bacteria-rich food sources, and are potential reservoirs for pathogenic bacteria of scientific importance.

Background Information

- ***Escherichia coli* O157:H7 found on fly 13 days later (Wasala *et al.* 2013)**
  - Fly surfaces beneficial for survival or growth of bacteria
- **Fly cleaning behavior reduce bacterial disease contamination (Jacques *et al.* 2017)**
  - Quantity of bacteria removed dependent on time after exposure
  - Different fly species remove specific microorganisms

OVERALL GOAL: *Understanding how bacterial communities fluctuate on flies and their animal hosts over time.*

Objectives

Using Illumina 16s rRNA sequencing:

- Compare bacterial communities of rat exposed flies to communities on flies
- Identify changes in those bacterial communities over time
- Investigate presence of pathogenic bacteria in sarcophagid fly interactions

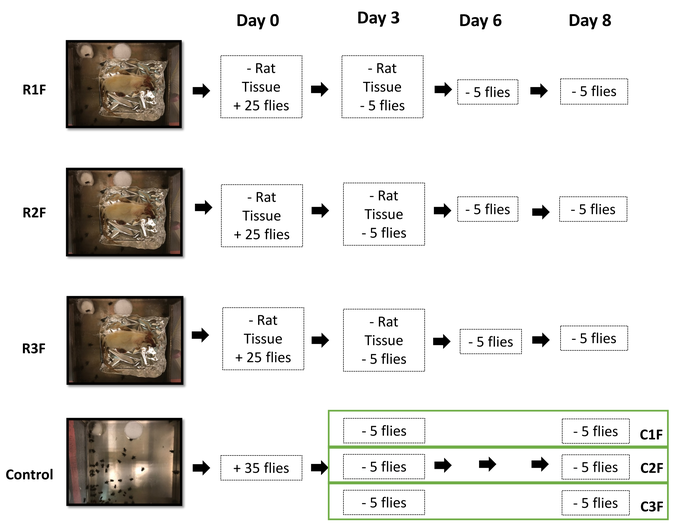

Figure 1: Protocol for collection of fly and rat samples.

Methods

Collection of Samples (Shown in Figure 1)

- 3 rats were decomposed in sealed containers for 1 week
- Samples of lower abdominal tissue collected from rats and stored in -80 C freezer
- Each rat added into a sterile cage with water and sucrose; 25 flies added into each cage
- 35 flies added into a "control" cage with just sucrose and water
- Day 3, rats removed from cages and lower abdominal tissues again collected and stored; 5 flies from each cage holding the rats, and 15 flies (three groups of 5) from control cage
- Day 6, 5 flies collected from each cages formerly holding rats; no control flies collected
- Day 8, 5 flies collected from each cage formerly holding rats, and 15 flies (three groups of 5) collected from control cage

Sample Preparation for Sequencing

- Prepared samples in 1/100 mass to buffer solution
- Ground flies and rat tissues in stomacher bags (10 seconds) & orbital shaker (30 minutes)
- 15 mL of sample centrifuged & pelleted
- Re-suspended pellet and extracted 500 µL for shipment

Spread Plate Dilutions

- 200 µL of re-suspended pellet extracted for dilutions
- Plated 1x10, 1x10^-1, 1x10^-2, 1x10^-3, 1x10^-4 dilutions for each sample
- Incubated at 37 C for 24 hours

Sequencing & Analysis

- **Omega Biosciences**
  - DNA Extraction, Library Preparation, & 16 rRNA
- **Kraken:** Assembly
  - Identified sequences and quantities of populations
- **Krona:** Analysis
  - Created pie charts from Kraken identifications
- **Diamond:** Identification
  - Precise reassignment of taxonomy

Data Analysis

- Top 5 bacterial genera on the flies and on the rats
- World Health Organization (WHO) "Global priority list of antibiotic-resistant bacteria…" (World Health Organization, 2017)
- Selected bacteria of interest

Results

Spread Plate Dilutions

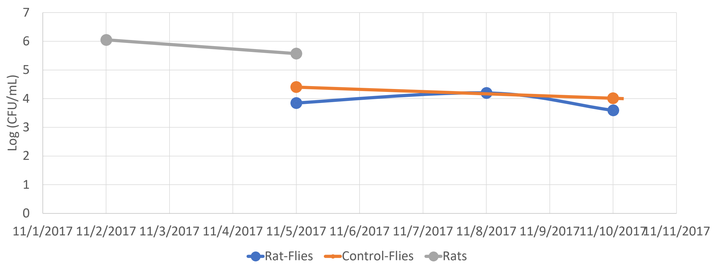

Figure 2: Log of average colony forming units (CFU) for flies exposed to rats, control flies, and rat tissues at each time point.

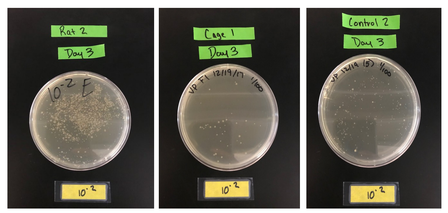

Figure 3: Spread plates for 10^{-2} mL dilutions of rat 2, control flies 2, and flies exposed to rats 2 after 3 days.

Bacterial Genera of Samples

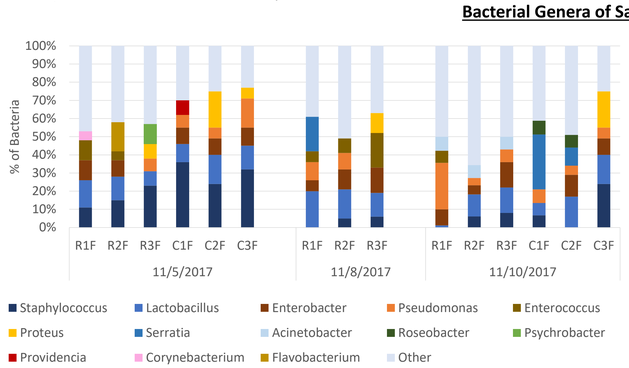

Figure 4: Five largest bacterial genus populations on fly samples over testing period.

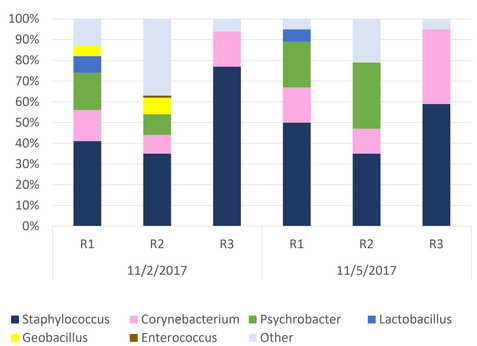

Figure 5: Five largest bacterial genera populations for rat samples over testing period.

WHO Priority List

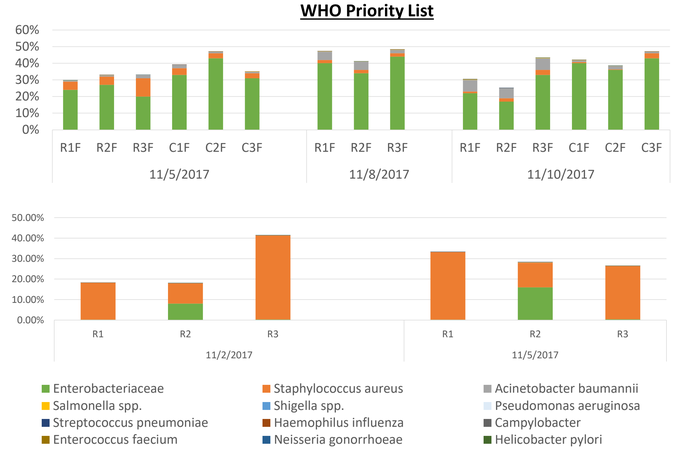

Figure 6: Percentages of WHO antibiotic-resistant bacteria in fly and rat samples over the 8 day testing period.

Table 1. Mean number of hits for four fly-associated bacteria recovered from flies caged with rotting rat carcasses at Day 3, Day 6 and Day 8

	Day 3	Day 6	Day 8
Providencia spp	776	321	90
Proteus spp	1786	2187	614
Klebsiella spp	30	40	18
Hafnia spp	17,661	23,518	22, 490

Discussion

From the plating experiment, cultivable bacteria decreased over time
From the 16s sequencing data:

- -16s analysis identified > 60 bacterial genera on rat-exposed flies
- -At least 11 of these genera were on the WHO 2017 Priority List
- -There was lower bacterial diversity on rats compared to flies. This may be because a small area of rat tissue was sampled vs the entire fly
- -*Staphylococcus* spp was the largest population for all samples
- -Comparison of fly-associated bacteria showed that *Providencia* and *Proteus spp* declined over time

Significance & Future Work

Significance

- Fly feeding behavior influences microbiome populations
- Inoculation of bacteria more natural than induced inoculation studies for microbial transmittance, better representing the natural relationships between the microorganisms of flies and their food sources

Future Work

- Further investigate targeted bacteria from data to better understand relationships of the fly with more bacteria
- Repeat experiment with other filth flies for comparisons between microbiomes

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