Evaluating the Role of Diet, Taxonomy, Sex, and Geography of the Oral and Rectal Microbiome of Puerto Rican Bats

Collaboration with Dr. Michael Willig

Ahmad Hassan

2019-11-08
Outline

• Background
• Why study bats?
• Methods
• Results
• Summary
“Evolution of Mammals and Their Gut Microbes”

Ley et al. (2008)
Background

Facts
• Only mammals capable of true flight
• Bats account for 20% of mammalian species
• They are found on every continent except Antarctica

Ecological and Economical Significance
• Bats act as natural pest control saving agriculture industry~ $3.7 billion per year
• Natural pollinators and seed dispersers
• Guano droppings are rich natural fertilizers

❖ White-nose syndrome: fungal infection caused by Pseudogymnoascus destructans that killed millions of bats in North America
Puerto Rican Bats

Why study bat microbiome?

• Harbor zoonotic pathogens
• Microbiome is understudied & poorly characterized
• Studying their microbiomes could help us answer:

❖ What factors could shape the microbial communities in bats?
• Factors that influence the composition of microbiome:
  • Diet
  • Host taxonomy
  • Sex
  • Geography

https://www.google.com/search?biw=1275&bih=681&tbm=isch&q=artebius+jamaicensis
Previous Research Suggests:

• Diet and host taxonomy have greatest effect on the fecal microbiome (Lee et al., 2008; Muegge et al. 2001).

• Most bat microbiome studies characterized the fecal microbiome and showed intra-species variation in microbiome (Banskar S. et al., 2016).

• Our study design differs by collecting oral and rectal swabs, while most other studies focused on the fecal microbiome.
Sample Collection Done by the Willig Lab in Puerto Rico
Diets of Nine Bat Species Analyzed in this Study

**Frugivore:** Fruits
- *Artibeus jamaicensis*

**Euryphagous:** Fruits, Nectar, Insects
- *Brachyphylla cavernarum*
- *Erophylla sezekorni*

**Nectarivore:** Nectar
- *Monophyllus redmani*

**Insectivore:** Insects
- *Mormoops blainvillii*
- *Eptesicus fuscus*
- *Pteronotus portoricensis*
- *Pteronotus quadridens*

**Piscivore:** Fish
- *Noctilio leporinus*
**Methods**

Bats caught by harp traps and hand nets

Oral and rectal swabs collected

DNA extracted using DNeasy PowerSoil kit/QIAcube

**PCR Amplify V4 16S rRNA gene**

Sequence on illumina Miseq

**Amplified Sequence Variants (ASVs)**

- Decontam
- phyloseq, microbiome, and vegan
- Samples rarefied to 1,000 reads
- 134 oral samples
- 155 rectal samples
Samples Rarefied to 1,000 Reads

<table>
<thead>
<tr>
<th>Species</th>
<th>Oral</th>
<th>Rectal</th>
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<tbody>
<tr>
<td>Artibeus jamaicensis</td>
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Does diet influence the oral and rectal microbiome?
Family Level Microbial Community Relative Abundance of Rectal Samples

**Relative Abundance Rarefied to 1000 Reads**

- **Pasteurellaceae**
- **Corynebacteriaceae**
- **Clostridiaceae_1**
- **Mycobacteriaceae**
- **Staphylococcaceae**
- **Brevinemaetaceae**
- **Spirochaetaceae**
- **Nocardioaceae**
- **Caulobacteraceae**
- **Acidimicrobiaceae**
- **Rhodobacteraceae**
- **Pseudomonadaceae**
- **Helicobacteraceae**
- **Neisseriaceae**
- **Veillonellaceae**
- **Xanthomonadaceae**
- **Acetobacteraceae**
- **Lactobacillaceae**
- **Unknown_Family**
- **Brevibacteriaceae**
- **Dermabacteraceae**
- **Lachnospiraceae**
- **Ruminococcaceae**
- **Pseudonocardiaceae**
- **Enterobacteriaceae**
- **Enterococccaceae**
- **Carnobacteriaceae**
- **Planococcaceae**
- **Bacillaceae**
- **Moraxellaceae**
- **Sphingomonadaceae**
- **Aeromonadaceae**
- **Dermacoccaceae**
- **Corynebacteriales_Incertae_Sedis**
- **Salinisphaeraceae**
- **Other**

**Additional Families**

- **Mycoplasmataceae**
- **Cardiobacteriaceae**
- **Family_XI**
- **Desulfovibrionaceae**
- **Chitinophagaceae**
- **Micrococccaceae**
- **Rikenellaceae**
- **Actinomycetaceae**
- **Alcaligenaceae**
- **Acidimicrobiaceae**
- **Pseudomonadaceae**
- **Pseudonocardiaceae**
- **Other**

**Euryphagous**

- **A. jamaicensis**
- **B. cavernarum**
- **E. sezekorni**
- **M. redmani**
- **N. leporinus**
- **E. fuscus**
- **P. portoricensis**
- **P. quadridens**

**Frugivore**

- **Frugivore**

**Nectarivore**

- **Nectarivore**

**Piscivore**

- **Piscivore**

**Insectivore**

- **Insectivore**
Influence of Diet on the Microbiome

**NMDS Oral Samples**

**NMDS Rectal Samples**

PERMANOVA = 0.001  
$R^2 = 0.404$

PERMANOVA = 0.001  
$R^2 = 0.165$
Does host taxonomy affect the microbiome?
Influence of Host Family on the Microbiome

NMDS Oral Samples

PerMANOVA = 0.001
$R^2 = 0.237$

NMDS Rectal Samples

PerMANOVA = 0.001
$R^2 = 0.081$
Influence of Host Species on the Microbiome

NMDS Oral Samples

NMDS Rectal Samples

PERMANOVA = 0.001
$R^2 = 0.544$

PERMANOVA = 0.001
$R^2 = 0.226$
Diet and Host Taxonomy Effect on the Microbiome

- Diet contributed to 40.4% of variation in the oral microbiome and 16.5% in the rectal microbiome.

- Host species contributed to 54.4% of variation in the oral microbiome and 22.6% of variation in the rectal microbiome.
Most Prevalent Taxa in Five Species Analyzed

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</tr>
<tr>
<td><em>Noctilio leporinus</em></td>
<td>Piscivore</td>
</tr>
<tr>
<td><em>Eptesicus fuscus</em></td>
<td>Insectivore</td>
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Frugivore *Artibeus jamaicensis* Relative Abundance of Most Prevalent Taxa

Oral Samples Rarefied to 5,000 Reads

Rectal Samples Rarefied to 5,000 Reads
Euryphagous *Brachyphylla cavernarum*
Relative Abundance of Most Prevalent Taxa

Oral Samples Rarefied to 1,000 Reads

Rectal Samples Rarefied to 1,000 Reads
Nectarivore *Monophyllus redmani* Relative Abundance of Most Prevalent Taxa

**Oral Samples Rarefied to 1,000 Reads**

**Rectal Samples Rarefied to 1,000 Reads**
Piscivore *Noctilio leporinus* Relative Abundance of Most Prevalent Taxa

Oral Samples Rarefied to 1,000 Reads

Rectal Samples Rarefied to 1,000 Reads
Insectivore *Eptesicus fuscus* Relative Abundance of Most Prevalent Taxa

**Oral Samples Rarefied to 1,000 Reads**

**Rectal Samples Rarefied to 1,000 Reads**
How Do Sex and Geography Influence the Microbiome?

• Are there cave specific taxa and where are these taxa found, oral or rectal microbiome?

• Are there differences between male and female bat microbiomes?
## Presence of ASVs Across Three Caves

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<th>Aguas oral</th>
<th>Mata oral</th>
<th>Rio oral</th>
<th>Aguas rectal</th>
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Is there an Effect of Sex and Cave on the Microbiome

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<td>Cave</td>
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<td>P-value</td>
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<td>P-value</td>
<td>R²</td>
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<tr>
<td>Jaccard</td>
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<td>0.018</td>
<td><strong>0.001</strong></td>
<td>0.096</td>
<td><strong>0.039</strong></td>
<td>0.089</td>
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Is there an Effect of Sex and Cave on the Microbiome

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<tr>
<td>Sex</td>
<td>Cave</td>
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<td>$R^2$</td>
<td>P-value</td>
<td>$R^2$</td>
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<tr>
<td>Bray Curtis</td>
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<td><strong>0.042</strong></td>
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<td><strong>0.001</strong></td>
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<tr>
<td>Jaccard</td>
<td>0.093</td>
<td>0.025</td>
<td><strong>0.044</strong></td>
<td>0.054</td>
<td><strong>0.011</strong></td>
<td>0.113</td>
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Bray Curtis 0.109 0.027 **0.042** 0.059 0.001 0.146 0.731 0.028 0.266 0.268 **0.044** 0.155 0.054 0.251 0.251 0.164

Jaccard 0.093 0.025 **0.044** 0.054 0.011 0.113 0.623 0.033 0.266 0.244 **0.035** 0.136 0.059 0.234 0.201 0.156
Summary

• Host species and diet had greatest effect on the oral microbiome.

• Only *B. cavernarum* and *N. leporinus* showed a difference between male and female microbiome.

• Geography had minimal effect on the microbiome. There was a significant difference in the microbiome of *A. jamaicensis* among three caves.

• Our results show a greater effect of diet and host taxonomy than previous studies probably because we used oral and rectal swabs.
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Dr. Jacquelynn Benamino  
Dr. Jeremiah Marden  
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Susan T. Janton

**Michael Willig Lab**
Dr. Michael Willig  
Dr. Steven Presley  
Anna R. Sjodin

This study was funded by UConn Provost Microbiome Research Excellence Award
Differential Abundance - Rectal Vs Oral

![Graph showing differential abundance between Rectal and Oral samples for various bacterial families and phyla. The x-axis represents the bacterial family, and the y-axis represents the log2FoldChange. The graph includes data points for Proteobacteria, Firmicutes, Spirochaetae, Actinobacteria, Tenericutes, and Fusobacteria.]
Skin Microbiome Can Aide in the Fight Against *Pseudogymnoascus destructans*

Bacteria Isolated from Bats Inhibit the Growth of *Pseudogymnoascus destructans*, the Causative Agent of White-Nose Syndrome

Joseph R. Hoy, Tina L. Cheng, Kate E. Langwig, Mallory M. Hee, Wintfred F. Frick, A. Mann Kilpatrick

Published: April 8, 2015 • https://doi.org/10.1371/journal.pone.0121329

Abstract

Emerging infectious diseases are a key threat to wildlife. Several fungal skin pathogens have recently emerged and caused widespread mortality in several vertebrate groups, including amphibians, bats, rattlesnakes and humans. White-nose syndrome, caused by the fungal skin pathogen *Pseudogymnoascus destructans*, threatens several hibernating bat species with extinction and there are few effective treatment strategies. The skin microbiome is increasingly understood to play a large role in determining disease outcome. We isolated bacteria from the skin of four bat species, and co-cultured these isolates with *P. destructans* to identify bacteria that might inhibit or kill *P. destructans*. We then conducted two reciprocal challenge experiments *in vitro* with six bacterial isolates (all in the genus *Pseudomonas*) to quantify the effect of these bacteria on the growth of *P. destructans*. All six *Pseudomonas* isolates significantly inhibited growth of *P. destructans* compared to non-inhibitory control bacteria, and two isolates performed significantly better than others in suppressing *P. destructans* growth for at least 35 days. In both challenge experiments, the extent of suppression of *P. destructans* growth was dependent on the initial concentration of *P. destructans* and the initial concentration of the bacterial isolate. These results show that bacteria found naturally occurring on bats can inhibit the growth of *P. destructans in vitro* and should be studied further as a possible probiotic to protect bats from white-nose syndrome. In addition, the presence of these bacteria may influence disease outcomes among individuals, populations, and species.
Frugivorous Females Feed on Insects During Pregnancy
Why Rarefy to 2000 Reads
“Rates of gut microbiome divergence in mammals”

Rarefaction Curve

5000 Reads

Species

Sample Size
Alpha Diversity-Oral Samples
Alpha Diversity-Rectal Samples

![Box plot showing alpha diversity measure across different diets](image)

- **Diet Categories:**
  - Euryphagous
  - Frugivore
  - Insectivore
  - Nectarivore
  - Piscivore

The box plot displays the observed range for each diet category, with outliers marked as individual data points.
Family Level Microbial Community Relative Abundance of Rectal Samples

Relative Abundance Rarefied to 1000 Reads

| Family Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name | Genus Name |
|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| A. jamaicensis | Pasteurellaceae | Helicobacteraceae | Neisseriaceae | Veillonellaceae | Xanthomonadaceae | Acetobacteraceae | Lactobacillaceae | Unknown_Family | Brevibacteriaceae | Dermabacteraceae | Lachnospiraceae | Ruminococcaceae | Pseudonocardiae | Other |
| B. cavernarum | Helicobacteraceae | Neisseriaceae | Veillonellaceae | Xanthomonadaceae | Acetobacteraceae | Lactobacillaceae | Unknown_Family | Brevibacteriaceae | Dermabacteraceae | Lachnospiraceae | Ruminococcaceae | Pseudonocardiae | Other |
| E. sezekorni | Pasteurellaceae | Helicobacteraceae | Neisseriaceae | Veillonellaceae | Xanthomonadaceae | Acetobacteraceae | Lactobacillaceae | Unknown_Family | Brevibacteriaceae | Dermabacteraceae | Lachnospiraceae | Ruminococcaceae | Pseudonocardiae | Other |
| M. redmani | Pasteurellaceae | Helicobacteraceae | Neisseriaceae | Veillonellaceae | Xanthomonadaceae | Acetobacteraceae | Lactobacillaceae | Unknown_Family | Brevibacteriaceae | Dermabacteraceae | Lachnospiraceae | Ruminococcaceae | Pseudonocardiae | Other |
| N. leporinus | Pasteurellaceae | Helicobacteraceae | Neisseriaceae | Veillonellaceae | Xanthomonadaceae | Acetobacteraceae | Lactobacillaceae | Unknown_Family | Brevibacteriaceae | Dermabacteraceae | Lachnospiraceae | Ruminococcaceae | Pseudonocardiae | Other |
| E. fuscus | Pasteurellaceae | Helicobacteraceae | Neisseriaceae | Veillonellaceae | Xanthomonadaceae | Acetobacteraceae | Lactobacillaceae | Unknown_Family | Brevibacteriaceae | Dermabacteraceae | Lachnospiraceae | Ruminococcaceae | Pseudonocardiae | Other |
| P. portoricensis | Pasteurellaceae | Helicobacteraceae | Neisseriaceae | Veillonellaceae | Xanthomonadaceae | Acetobacteraceae | Lactobacillaceae | Unknown_Family | Brevibacteriaceae | Dermabacteraceae | Lachnospiraceae | Ruminococcaceae | Pseudonocardiae | Other |
| P. quadridens | Pasteurellaceae | Helicobacteraceae | Neisseriaceae | Veillonellaceae | Xanthomonadaceae | Acetobacteraceae | Lactobacillaceae | Unknown_Family | Brevibacteriaceae | Dermabacteraceae | Lachnospiraceae | Ruminococcaceae | Pseudonocardiae | Other |

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Artibeus jamaicensis - ASVs across three caves

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