Environmental and host factors affect bacterial community structure in the mosquito gut

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Introduction
Insect species host many microbial symbionts.
- Mosquitoes acquire a gut microflora by ingesting bacteria in their larval aquatic environment.
- These bacteria are necessary for larva to develop to adulthood (Coo et al. 2014).
- This larval bacterial community persists into adulthood and is important for maintaining mosquito health by aiding in nutrient acquisition and reproduction (Minnard et al. 2013).
- By investigating the bacterial communities in three species of mosquitoes, we assessed how environment x species interactions affect community composition.
- We had two main objectives:
  1. Characterize the gut bacterial communities that develop in Aedes aegypti, Aedes albopictus, and Culex quinquefasciatus when reared separately.
  2. Characterize the gut bacterial communities of these species when reared together.

Background
Bacteria in the genera Chrysobacterium, Microbacterium, and Comamonas are dominant members of the A. aegypti gut community.

<table>
<thead>
<tr>
<th>Proportion of reads</th>
<th>0</th>
<th>0.5</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td></td>
<td></td>
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<tr>
<td>Larvae</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Actinobacteria</th>
<th>Gamma-proteobacteria</th>
<th>Beta-proteobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacteriaceae</td>
<td>Aeromonadaceae</td>
<td>Rhodobacteraceae</td>
</tr>
<tr>
<td>Microbacteriaceae</td>
<td>Xanthomonadaceae</td>
<td>Oxalobacteraceae</td>
</tr>
<tr>
<td>Myxococcales</td>
<td>Monocloaceae</td>
<td>unknown Beta-proteobacteria</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Pseudomonadaceae</td>
<td>Nocardiaceae</td>
</tr>
<tr>
<td>Comamonadaceae</td>
<td>Comamonadaceae</td>
<td></td>
</tr>
</tbody>
</table>

Mosquito larvae reared in the laboratory. Data were generated by high-throughput sequencing of 16S rRNA. Phylum and family names are provided. Each bar indicates the proportion of reads that matched the given bacterial family. Only categories >2% are shown. Shown boxed in red are three dominant families from which bacterial strains were selected to perform experiments with artificially structured communities. From Ralobacteraceae, Chrysobacterium; from Microbacteriaceae, Microbacterium; from Comamonadaceae, Comamonas. Figure adapted from Coo et al. (2014).

Methods
Produce bacteria-free (axenic) mosquito larvae
Add axenic larvae to dishes containing sterile water and diet
Inoculate with bacterial isolates of interest
Characterize relative abundance of each bacterial isolate in fourth instar larvae

Results
1. The initial composition of bacteria in the aquatic environment does not affect the final bacterial community in the larval gut
   - A: Starting concentration of bacteria in the water
     - 1:1
     - CWM
     - INV
   - B: Final concentration of bacteria in A. aegypti larvae
     - 1:1
     - CWM
     - INV
   - C: Final concentration of bacteria in A. albopictus larvae
     - 1:1
     - CWM
     - INV
   - D: Final concentration of bacteria in C. quinquefasciatus larvae
     - 1:1
     - CWM
     - INV

2. Cohabitation with another species of mosquito does not affect the gut bacterial community
   - A: A. aegypti
     - C. quinquefasciatus
     - Bacterial composition
   - B: A. albopictus
     - C. quinquefasciatus
     - Bacterial composition

Relative abundance of three dominant community members in fourth instar larvae measured by qPCR. Each dish was inoculated with a 1:1 ratio mixture of the three bacterial isolates of interest. In experiment A, A. aegypti and C. quinquefasciatus were reared together and in experiment B, A. albopictus and C. quinquefasciatus were reared together. The final community in C. quinquefasciatus differed from both A. aegypti and A. albopictus even when reared in the same environment (p < 0.0001).

Conclusions
- Community structure in larvae is robust to changes in the environment.
- Closely related mosquito species have similar gut communities.
- This indicates that both the environment and the larval host play an important role in the development of the gut community.
- Future studies will focus on identifying physiological factors underlying host determination of gut bacterial communities.

References

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