A Tropical Microbial Observatory: Collaborative research on microbial diversity in caterpillars

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A Microbial Observatory was established at the Area de Conservacion Guanacaste, Costa Rica (http://alrlab.pdx.edu/research/mocat/index.html) to sample, inventory, quantify, monitor and culture the microbiota (Archaea, Bacteria, Eukarya) associated with caterpillars (including the intestine, frass and associated plant material) feeding on a single tree species (*Spondias mombin*). Our goal is to relate functions of this microbiota to caterpillar biology and ecological roles in a tropical forest ecosystem.

The overall pattern of microbial diversity was explored by T-RFLP analysis. Over 713 DNA samples have been screened by T-RFLP of 18S/16S rDNA and the results have been analyzed by one-way ANOVA. Initial results suggest there is no association of bacterial diversity with caterpillar life stage or plant host, but the results are preliminary, as the dataset is skewed to early larval instars.

Culture-based studies revealed a diversity of eukaryotes and prokaryotes. A Heteromita-like flagellate was isolated from a *Rothschildia lebeau* (Saturniidae) larval gut. Microscopic observations of glass cover-slip incubations of frass material from *R. lebeau* revealed both flagellates and ciliates, and a colpodid ciliate was isolated. Acanthamoebae were observed from Spondias leaf washes. Acanthamoebid, myxomycete, and vannellid amoebae from leaf wash inoculations are being characterized in culture. The major fungal genera cultured were all general leaf-surface and soil fungi (*Penicillium, Aspergillus, Aureobasidium, Alternaria,Cladosporium, Fusarium and Hyphomycetes*). Of the 356 aerobic heterotrophic bacterial isolates cultured, (Proteobacteria, Firmicutes, Actinobacteria and Bacteriodetes), the majority were related to common soil microbes. Based on pH and 0_2 measurements in *R. lebeau* gut partitions, the midgut was consistently pH 9 to 11 and low in 0_2, while the hindgut was pH 4 to 7 and varied in 0_2 concentration. Given these constraints, we enriched for alkalophilic bacteria and eukaryotes and obtained enrichments dominated by Enterobacteriaceae and close relatives of *Enterococcus flavens*.

Molecular phylogenetic analysis of clone libraries revealed colpodid and cercozoan protists on *S. mombin*. Sequences were also recovered with high similarity to vannellid lobose amoebae, myxomycetes, and basidiomycete fungi. No protist or fungal sequences were amplified directly from guts (> 300 samples), however. In contrast, molecular analysis of PCR-amplified 16S rDNA from larval midguts (236 clones analyzed) revealed significant overlap with bacterial groups found in aerobic cultures, although a number of phylotypes were not represented by cultured isolates. Examples of the latter include numerous clones affiliated with members of the Acidobacteria and uncultured environmental sequences that diverged from cultured *Bacillus* species.

To explore the genomic diversity of microorganisms associated with caterpillars, we have also initiated a metagenomic approach. We used *Manduca sexta* as a model to optimize protocols for sampling, bacterial cell separation, storage and transport, extraction and cloning of large DNA fragments for construction of metagenome libraries from larval midgut-associated microorganisms. Our methods enriched for large fragments of clonable bacterial DNA, but clone numbers were limited by the low concentrations of purified microbial DNA recovered from larvae. Further characterization of the DNA inserts is underway. Work in progress will compare recovery of microbial phylotypes using total midgut DNA extraction and amplification of 16S rDNA to that from bacterial cell separations and direct cloning into large-insert metagenome libraries.