

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, Eukaryotes) associated with caterpillars (including the intestine, frass and associated plant material) feeding on the native tree species *Spondias mombin*. Our goal is to relate functions of this microbiota to caterpillar biology and ecological roles in a tropical forest ecosystem. Central to this goal is identification of resident, vs. transient, gut microbiota. This question is being approached, in part, through comparison of microbial populations from the midguts of caterpillars fed either an artificial or natural diet.

Patterns of microbial diversity associated with caterpillar guts were also explored by T-RFLP analysis. A web-based tool was developed to automatically analyze T-RFLP datasets. Over 700 DNA samples were screened by T-RFLP of 18S/16S rDNA and the results analyzed by one-way ANOVA. Analysis by the Kruskal-Wallis and the Wilcoxon Rank Sum tests revealed some differences in the gut microbiota that were dependent upon host plant species and caterpillar life stage.

Culture-based studies revealed a diversity of eukaryotes and prokaryotes from the guts and frass of *Rothschildia lebeau* (Saturniidae) larvae; isolates include a Heteromita-like flagellate and a colpodid ciliate. Acanthamoebae were observed from *S. mombin* leaf washes, and 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, and the majority of 356 aerobic heterotrophic bacterial isolates were also related to common soil microbes. Enrichments based on measurements of pH (9 to 11) and O₂ (low concentrations) in the caterpillar midgut were dominated by Enterobacteriaceae and close relatives of *Enterococcus flavens*.

Molecular phylogenetic analysis of eukaryotic clone libraries revealed colpodid and cercozoan protists on *S. mombin*, and sequences with high similarity to vannellid lobose amoebae, myxomycetes, and basidiomycetes. Sequence analysis also revealed significant differences in clone libraries constructed from leaf washes of dry and wet forest trees, but no protist or fungal sequences were amplified directly from guts. 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 diverge from cultured *Bacillus* species. Given the low pH (<4.0) from *S. mombin* leaf washes, the recovery of 16S rDNA representing alkaliphilic microorganisms suggests they may colonize the caterpillar midgut. Consistent with this idea, a dominant bacterial isolate from *R. lebeau* larval midguts grew at pH 7 with both high and low concentrations of O₂, but at pH 10.5 under conditions of low O₂ only.

The small amount of microbial biomass recovered from the midgut (approximately 10⁵ cells/gut) using a cell-separation technique presented significant challenges for construction of metagenome libraries. End sequencing of randomly selected metagenomic clones, however, demonstrated that libraries constructed using this approach were enriched for bacterial DNA. Sequence-based screens indicate that the inferred microbial populations represented by gene sequences from the metagenome libraries, and the SSU rDNA libraries made from direct extraction of whole midgut samples, together demonstrate the presence of diverse and unique microorganisms associated with caterpillar midguts. Ongoing research will further delineate which members of these populations are caterpillar midgut residents.

