The Identification and Characterization of Bacterial Endosymbionts in the Planthopper family, Caliscelidae(Sternorrhyncha: Fulgoroidea)

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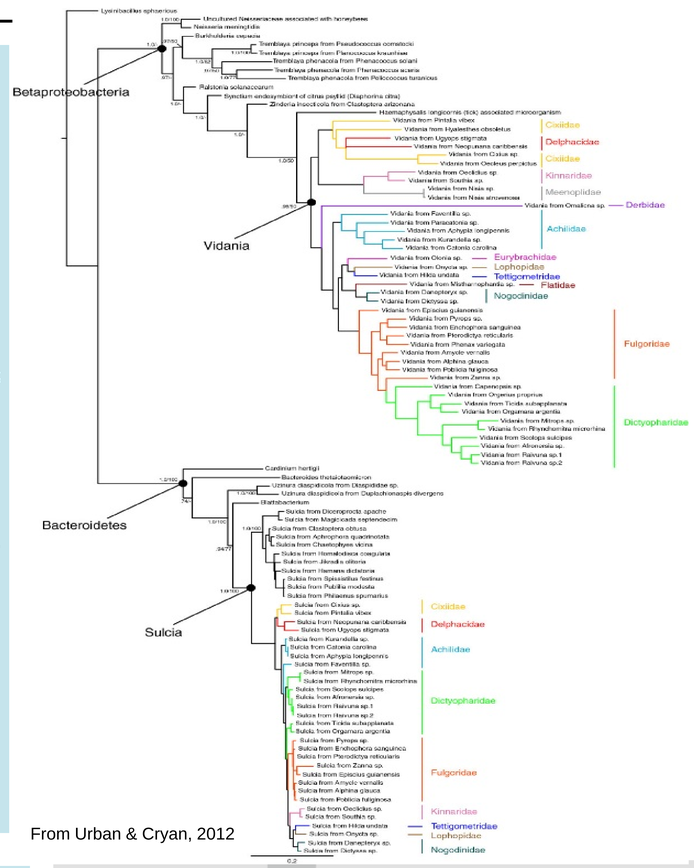
Abstract

Plant-sap sucking insects with deficient nutritional diets contain symbiotic microorganisms in a specific organ in the abdomen (bacteriome). Such symbionts were discovered to belong to various bacterial lineages. Ongoing genomic research concluded that these symbiotic organisms provide the essential nutrients that are absent in the insect's diets such as amino acids and vitamins. While the identities and functions of several sap-sucking lineages have been studied, some lineages such as Fulgoroidea are not understood because recent results failed to confirm the presence of the symbiotic microbes even though older microscopy studies identified microbes in the bacteriomes. The objective of this research is to learn methods that will aid in identifying the bacterial symbiotic diversity in Caliscelidae by using several techniques such as: dissection, DNA extraction, PCR 16S gene amplification, TA subcloning, bacterial plating, colony screening, sequence assembly, and Genbank queries. Such methods will be put to use to amplify sequence and identify the bacterial endosymbionts in Caliscelidae. We will then determine the symbionts' relationships to other known insect symbionts and free living bacteria. We are expecting to find unique 16S sequences that would represent the projected diversity of bacterial endosymbionts. Our results will help us to determine how closely related these symbionts are to those in other planthopper families. Our further goal is to learn cryosectioning techniques and *in situ* hybridization methods with specific probes, which will be used to localize sequences amplified from putative symbionts to the bacteriome, thereby confirming that our sequenced DNA came from the symbionts residing in the bacteriome.



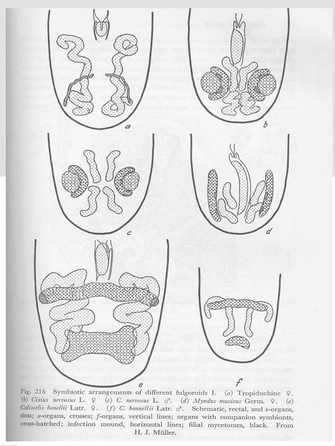
From Urban & Cryan, 2012

Hypothesis

Older microscopy studies have identified microbes such as *Sulcia* and *Vidania* in the bacteriomes of Caliscelidae but recent studies have failed to confirm their presence. We hypothesize that these microbes are present and we will show to which degree they are related to those of other groups in the planthopper lineage.

Introduction

Symbiotic relationships have been under continuing research for hundreds of years. Regardless of the amount of research that has been done and the data that have been collected, some symbionts have not been fully understood. Plant-sap sucking insects such as Caliscelidae are the best candidates to demonstrate such relationships due to their deficient nutritional diets and their special compartments that contain the symbiotic organisms which were discovered to belong to some bacterial lineages. Ongoing genomic research concluded that these symbiotic organisms do indeed provide the essential nutrients that are absent in the insect's diets. such as amino acids and vitamins. What's puzzling about this unique lineage is that recent studies have failed to identify the presence of these symbiotic microbes even though they have been identified by older microscopy studies.



From Müller, H.J. 1940

Expected Results

Identify unique 16S sequences demonstrating the diversity of the bacterial endosymbionts in Caliscelidae The symbionts, *Sulcia* and *Vidania,* will be compared to determine how closely related they are to those of other planthopper families, or whether they represent novel associations.

Future Goals

Learn cryosectioning techniques in situ hybrid-ization methods with labeled specific probes

References

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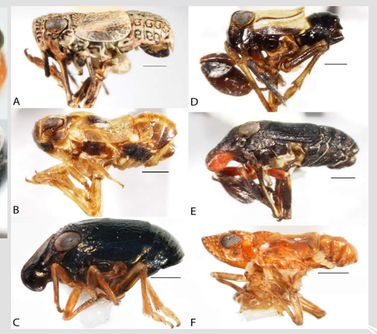
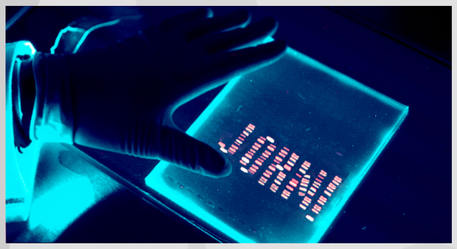
2. Moran, N. A. and G. M. Bennett (2014). The Tiniest Tiny Genomes. Annu. Rev. Microbiol. 68:195-215.
3. Müller, H.J. 1940a. Die symbiose der Fulgoroiden (Homoptera: Cicadina). Zoologica 98(1):1–110.
4. Müller, H.J. 1940b. Die symbiose der Fulgoroiden (Homoptera: Cicadina). Zoologica 98(2):111–220.
5. Urban, J. and J. Cryan (2012). Two ancient bacterial endosymbionts have coevolved with the planthoppers (Insecta: Hemiptera: Fulgoroidea). BMC Evolutionary Biology 12(1): 87.

Methods

Obtain and dissect samples of Caliscelidae



Amplify the bacterial symbiont gene with PCR with a thermocycler



Run the amplified DNA from PCR on an agarose gel alongside a DNA ladder to help compare the length of the DNA

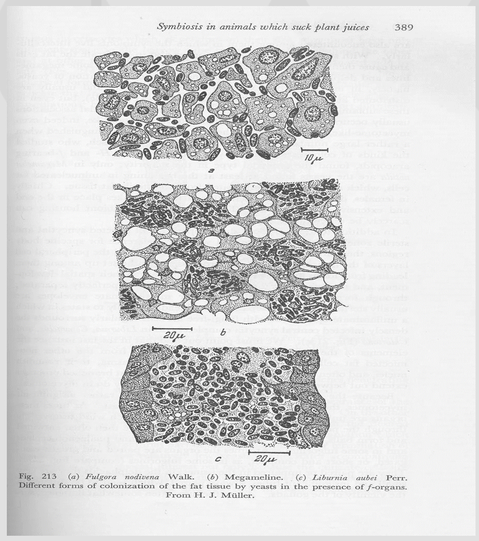
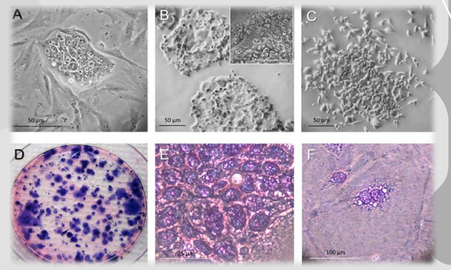
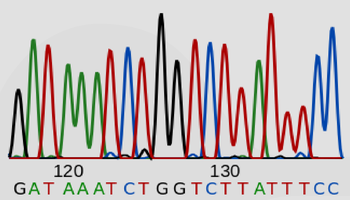


Fig. 213 (a) *Fulgora nodicosta* Walk. (b) Megamelinæ. (c) *Liburnia aubei* Perr. Different forms of colonization of the fat tissue by yeasts in the presence of *f*-organs. From H. J. Müller.



Sub-clone the PCR product then send off for sequencing

Assemble and query the sequence in Genbank using BLAST



Assemble bacterial 16S gene that includes the new sequences, to help analyze the phylogenetic relationship of the species with other known insect symbionts and free-living bacteria

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