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Techniques for conducting the bean beetle microbiome project on eggs

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Extended Abstract

The Bean Beetle Microbiome Project (www.beanbeetles.org) provides a set of class-tested protocols that permit undergraduates to conduct original research. Using a course-based undergraduate research experience (CURE) approach, students explore the bacterial microbiome communities found in the digestive tract of bean beetles, *Callosobruchus maculatus*. Female bean beetles attach individual fertilized eggs to the surface of the host bean in which their offspring feed, develop, and pupate. We have developed a simple and reliable method for students to obtain bean beetle eggs to conduct bacterial microbiome studies. Individual fertilized adult female bean beetles were introduced to a small number (4-5) of sterile 5 or 6mm glass beads in a 35mm sterile Petri dish. Females readily laid eggs on these glass beads. The collection of glass beads with eggs (at least 5 eggs total) from one female provided enough material to either extract the bacterial microbiome for culturing or to perform DNA extraction for diversity assay sequencing of the V4 region of the 16S rRNA bacterial gene. Eggs laid on glass beads cannot develop beyond the embryonic and first instar larval stage since they cannot burrow into a substrate, and they are prevented from feeding. We surface sterilized the beads and eggs in the same manner as adult beetles (Cole et al., 2018) by placing the beads in a cell strainer cup before dipping through a sequence of bleach, sterile water, ethanol, and then sterile water. A sterile 2mL microfuge tube containing 4-5 glass beads with saline (for culture plating) or the first extraction buffer (for DNA extraction) placed in a pulsing vortex machine for 60 seconds will crush the eggs and strip the eggs from the beads. This technique has permitted our students to successfully perform studies comparing the bacterial microbiomes of virgin adult bean beetles to eggs and embryos.

One of the challenges in attempting to extract microbiome samples from such limited substrates as insect eggs is the very limited amount of bacterial cells and DNA that can be obtained. Obviously, more eggs would result in more bacteria and DNA that could be extracted, but using eggs from the same female for a given sample has the benefit of ensuring that each sample is independent. Thus, a minimum of 5 eggs are needed in each sample, but more is better. It is also essential that a negative control is prepared since there is always contamination and even the DNA extraction reagents contain bacterial contamination.

The data shown in the poster were based on using the pulsing vortex for 5 minutes, but this long vortex interval is unnecessary. We evaluated shorter time intervals to judge whether the eggs were fully stripped from the beads and homogenized. We also evaluated 10 second, 30 second and 60 second pulsing vortex durations. The 60 second time best achieved both outcomes and has the benefit of minimizing the production of heat, bead shattering and mechanical failure of the pulsing vortex unit. The quality of extracted DNA (based on the shape of the absorption curves) was best at 60 seconds, but the concentrations were not significantly different for any of the pulsing vortex

durations.

We used glass beads for this project because they can be steam sterilized prior to use. However, once eggs are laid on beads, the first step is to surface sterilize the beads again. Thus, other substrates may be successfully substituted for glass beads. We have previously observed bean beetles readily lay eggs on plastic and wood beads, but these substrates would be more challenging to sterilize prior to use.

CITED REFERENCES

Cole MF, Acevedo-Gonzalez T, Gerardo NM, Harris EV, Beck CW. 2018. Effect of Diet on Bean Beetle Microbial Communities. Article 3 In: McMahon K, editor. Tested studies for laboratory teaching. Volume 39. Proceedings of the 39th Conference of the Association for Biology Laboratory Education (ABLE). http://www.ableweb.org/volumes/ vol-39/?art=3

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