Instructor's Notes on Student Preparation of Microbiome for Plating

The following are the lists of equipment, supplies and preparation instructions for microbiome collection and plating that your students will perform once beetles are available from your experiment. The quantities listed are those needed for a class of 24 with students working in 6 groups of 4 students. We are assuming that each group will evaluate the microbial community from two beetles - one from each of two experimental treatments. The quantities per group would need to be increased if the number of treatments were greater than two.

Equipment

- Test tube racks for 15mL conical tubes - 1 per group, 6 total
- Microfuge racks - 1 per group, 6 total
- Bunsen burner and striker-lighter – 1 per group, 6 total
- Glass petri dishes with 70% ethanol for spreader dipping– 1 per group, 6 total
- Microfuge – 1 per group, 6 total
- Micropipetters P20 – at least 1 per group, 6 total
- Micropipetters P200 – at least 1 per group, 6 total
- Rack for Micropipetters – 1 per group, 6 total (optional)
- Cell spreader (steel or glass) – 1 per group, 6 total
- Sterile pestles for 1.5mL microfuge tubes - 3 per group, 18 total
- Soft forceps for handling beetles – 1 per group, 6 total

Supplies

- Disposable gloves
- Nutrient agar (NA) plates – 6 per group, 36 total
- Eosin Methylene Blue (EMB) – 2 per group, 12 total
- Phenylethyl alcohol agar (PEA) – 2 per group, 12 total
- Sterile pipette tips for P20 micropipetter – 1 box per group, 6 boxes
- Sterile pipette tips for P200 micropipetter – 1 box per group, 6 boxes
- Sterile 1.5mL microfuge tubes
- Sterile water (200mL) – dispensed in 2-15mL conical tubes per group, 12 total
- 70% ethanol (200mL) – dispensed in 1-15mL conical tubes per group, 6 total and for spreader dipping dishes
- 10% bleach (100mL) – dispensed in 1-15mL conical tubes per group, 6 total
- Sterile 0.9% saline (20mL) – dispensed in 2-1.5mL microfuge tubes per group, 12 total
- Autoclavable pouches, (if needed for sterile microfuge pestles) – 1 pouch per group, 6 total
- 35mm plastic petri dishes for isolating individual beetle

Bean Beetles

- Live cultures of the treatment groups for your experiment. Need a minimum of 6 live beetles from each treatment.

Detailed instructions for preparing media for NA, EMB and PEA plates is described in the Instructor's Notes on Pouring Plates.

Prior to class (two days) prepare the following:

Sterile 0.9% saline: 0.9g NaCl in 100mL distilled or deionized water. Stir until dissolved. Autoclave. Dispense in sterile 1.5mL microfuge tubes. Label the tubes.

70% ethanol: using denatured 95% lab grade ethanol, measure 140mL ethanol and add 60mL of distilled or deionized water. Dispense in sterile 15mL conical tubes. Label the tubes. Keep tightly closed. Dispense to spreader dipping glass petri dishes, one per group.

10% bleach: using standard supermarket laundry bleach, add 10mL bleach to 90mL of distilled or deionized water. Dispense in sterile 15mL conical tubes. Label the tubes.

Autoclave 200mL of distilled or deionized water. Dispense in sterile 15mL conical tubes. Label the tubes.

Assemble the tubes of bleach, ethanol and sterile water in test tube racks so each rack contains 1 tube of bleach, followed by 1 tube of water, followed by 1 tube of ethanol, followed by a second tube of water. Each group will get one of these racks.

If the microfuge pestles are new, they will be sterile and in individual wraps. These plastic pestles are autoclavable and reusable. If the pestles have been previously used, rinse with water, dry and package in autoclavable pouches, 3 pestles per pouch (only 2 are needed per group, the third is a back-up). Orient the pestles so the handle end is pointed toward the end of the pouch that is pulled apart to access contents. Autoclave prior to use.

Autoclave boxes of pipette tips and covered jars or beakers of microfuge tubes.

Important reminders for students

- If students are unfamiliar with the use of micropipetters, remind them (demonstrate) how to correctly set the volume and accurately pipette the volume they set.
- Ask your class to tell you what the negative control plate (on NA) should be. Using 100uL of sterile saline makes sense here.
- Sterile saline is used for the negative control, to prepare the full-strength bean beetle microbial extract, and to prepare the 1/10 concentration of the microbial extract.
- Be sure students do not confuse the sterile saline with the rinse waters used for surface sterilization of the beetles. The sterile rinse water will not remain sterile after beetles have been carried through the sequence of bleach-water-ethanol-water.
- Spreaders must be sterilized between spreads from different sources or different concentrations of microbial preparations.
- Remind students to clearly label every tube with the beetle ID number and the experimental treatment for the beetle from which they are collecting a microbiome. That same information needs to be written on each plate that receives that collected microbiome, as well as the names of the students. Initials and codes can become confusing. Students should write on the bottom of the plate, not the lid, so there will be no chance of the identity of a plate being confused.
- Remind students that the cell spreaders and microfuge pestles are expensive and reusable. Place a small beaker containing a few mL of 70% ethanol for students to place their used pestles and remind students to sterilize their cell spreader each time they are done using it and between samples.

- Remind students that they should pipette the microbiome extract onto their PEA plate last and spread that plate first because liquid is most quickly absorbed on that medium.
- Turn inoculated plates upside down prior to incubating them. Incubation may be at 37°C for 24 hours, then parafilm wrapping the plates and hold them in the refrigerator to prevent overgrowth. Alternatively, plates may be held at room temperature to incubate for a week. It is best not to use a gravity type oven-incubator with plastic plates.