Student Handout - Bean Beetle Microbiome Culturing Protocol

The method below is based on "Effect of Diet on Bean Beetle Microbial Communities" by Cole et al. to be published in *Tested Studies for Laboratory Teaching, Proceedings of the Association for Biology Laboratory Education,* Volume 39.

Beetles are sacrificed by freezing them at -80^{0}C for 5 minutes. Before collecting and plating the internal microbiota of the beetles, the outside of the beetles is sterilized to avoid growth of external or contaminating microbes.

- Using sterile tweezers to grasp the beetle, submerge it in the following solutions:
  - 10% Bleach for 3 seconds
  - Sterile water for 10 seconds
  - 70% ethanol for 5 seconds
  - Sterile water for 10 seconds
- Place the beetle in a sterile microtube and add 450µL 0.9% sterile saline solution.
- Using the sterile pestle, crush the beetle to release its interior microbiota.
- Pellet the large debris by spinning in a microfuge for 5 seconds.
- Plate 100µL of the microbiota solution on each of the following plate types
  - Nutrient agar (NA)
  - Eosin Methylene Blue (EMB) – selective for gram-negative bacteria
  - Blood agar (BA) – selective for fastidious bacteria
  - Phenylethyl alcohol agar (PEA) – selective for gram-positive bacteria
- The solution will be absorbed by the PEA plate quickly. So, spread this plate immediately after pipetting.
- Dilute 20µL of the remaining microbiota solution by mixing with 180µL of 0.9% sterile saline and plate 100µL of this onto an NA plate.
- Plate 100µL of your sterile saline solution on an NA, EMB, BA, and PEA plates to serve as a negative controls.

Label each plate (the bottom of the plate, not the lid) with the beetle ID, experimental treatment experienced by the beetle from which the microbiome was extracted, the type of medium, your group identification, and the date.

Incubate plates, upside down, for a minimum of 24 hours at 37°C.

Describe the unique microbial phenotypes that can observed on the plates and record the number of bacterial colonies with each phenotype for each plate. Use characteristics below (and on the next page) to describe the phenotypes:

Color: W (white), O (off-white), R (red), O (orange), Y (yellow), B (brown)
Gloss: S (shiny), M (matte)
Form: C (circular), I (irregular), F (filamentous), R (rhizoid)
Elevation: R (raised), C (convex), F (flat), U (umbonate), Cr (crateriform)

Colony Phenotype Traits

Reproduced from: Microbeonline, Medical Microbiology Guide, Colony Morphology of Bacteria; How to describe Bacterial Colonies?
https://microbeonline.com/colony-morphology-bacteria-describe-bacterial-colonies/

Bacteria grow on solid media as colonies. A colony is defined as a visible mass of microorganisms all originating from a single mother cell. Key features of these bacterial colonies serve as important criteria for their identification.

Color (pigmentation): Some bacteria produce pigment when they grow in the medium e.g., green pigment produces by *Pseudomonas aeruginosa*, buff colored colonies of *Mycobacterium tuberculosis* in **L.J medium**, red colored colonies of *Serratia marcescens.* We will categorize bacterial colonies as having one of the following colors: white, off-white, red, orange, yellow, or brown.

Gloss: Some colonies appear to have a **shiny** or glossy surface while others have a **matte** or non-glossy surface.