Exploring plants and soils:
Rhizobia cultivation

Summary:
Beneficial microbes are everywhere, one of the easiest place to locate these in a soil context are Rhizobia, a nitrifying bacteria (diazotrophs), that make roots its home. It inhabits the root nodules of the legume family (Fabaceae), external to a host the bacteria is unable to express genes that allow it to fix nitrogen. In return for offering a home and sugar as feed, the plant receives a reliable source of Nitrite, a macronutrient.

This activity allows participants to locate, extract and cultivate the bacterium located in these nodule. It makes something often taught as a concept, which is widely abundant yet somehow extremely inaccessible, easily available and possible to interact with.

Leanring Objectives:
- Exploring the needs of bacterium

- Observing the results of symbiotic relationships

- Testing the impact on symbiotic relationships in relation to development of plants

Equipment:
- Petri dish with nutrient agar x5

- Distilled water (boiled and cooled water also possible)

- Pipets

- 5-10% bleach

- Lighter/gas stove

- Tweezers

- Needle tips or more forceps

- Glass bowls

Preparation:
- Estimated time 20 minutes.

- Gathering legume roots with nodules, potentially may want to gather a number from various sites for comparison.

- Remove soil from roots in order to be able to examine

- If not purchasing premade petri dishes, make up dishes with nutrient agar and try to maintain sterility as much as possible.

Time Required:

Introduction 5 mins, introducing role of rhizobia and tasks
If gathering root materials and cleaning 20 mins
Preparing nodules for removal 5 mins
Extracting rhizobia 5 mins

Total timing 35 minutes.

Cultivation time 5-7 days.

Background Leanring Needs:

- Understanding of symbiotic relationships
- Understanding of bacterium
- Understanding of the nitrogen cycle and nitrifying bacteria's role

Risk Assessment:

Hazard	Liklihood	Severity	Mitigation
Illness from soil ingestion	Low	Medium	Use gloves when handling the soil
Illness from cultivated organisms in dish	Medium	Medium/High	Wear glove when needed
Site/local specific risks	Unknown	Unknown	Anyone running this activity is advised to conduct a risk assessment for the specific site and conditions

Description of Activities:

1. Use a brush to clean the soil from the roots of the legume plant, ensuring it has small white to pick nodules
2. Cut off leaf matter
3. Submerge roots into bleach for 5 minutes
4. Rinse root material in water
5. Use a clean surface to place the roots on and sterilize tweezers
6. Use tweezers to pull off a nodule
7. Add a drop of water to the centre of the agar
8. Place the nodule into the drop of water upon the agar, use the tweezers to crush the nodule- it should release a slightly cloudy liquid into the water drop
9. Place lid on dish and label dish with date
10. Place dish out of sunlight or cover, somewhere between 20-30oc
11. After 24 hours the milky solution released from the nodule begins to become white, it starts to form a large white slimey patch. Others may form but this is contamination of the agar.

Potential extension:

After ~10 days use a hooked probe or a teaspoon to gently scrape the white Rhizobia and add to water at 25oc, place in a bottle and shape well. This creates an

inoculant of Rhizobium; this can then be used in a bean root development experiment.
Place compost in the microwave for 10 minutes, this sterilizes it. Place in 2 seed trays when cool and sow runner beans. Water one with tap water and the other with the rhizobia inoculant, label.
Grow the beans for 3-4 weeks old and then remove plants, clean roots and examine the nodules formed.