**Macrophonima phaseolina Isolation Protocol for Plants**

1. **Preparation/Grinding**
   1. Separate root from stem at soil level. Store in paper bag
   2. Take 5-10 tap roots per sample and process in a Christy Mill Tissue grinder using a 1 mm sized sieve. Store in a paper bag.
   3. Weigh out 0.05g ground sample and place in a 2ml microcentrifuge tube
   4. Samples can be stored at room temperature until ready to process

* Prepare bleach solution before beginning isolation
  + 730ul NaOCl/99.3ml H2O
* Prepare media before beginning isolation
  + 50ml PDA (1.95g) in ≥200mL flasks
  + Autoclave and cool to 55˚C. Do not amend with 0.05g Rifampicin and 238ul Tergitol until step-i in the Isolation Protocol
    - Note: Tergitol is viscous, use wide orifice tips
    - Note: Run samples in sets of ≤5 to protect sample viability from bleach solution

1. **Isolation - Can begin as soon as media is finished autoclaving**
   1. Add 1 ml of prepared 0.6% NaOCl solution to each sample
   2. Vortex for 4 - 1 min intervals with 15 sec rest in between
   3. Centrifuge samples at 10,000 x g for 1 min
   4. Top off solution with sterile water, approx. 1ml
   5. Wash the tissue sample in a #325 sieve for 30 secs
   6. Collect washed sample in a 15ml tube getting as little water as possible in the tube (<6ml)
   7. Rinse sieve with alcohol and water after each use
   8. Weigh out 0.05g Rifampicin  
      Complete Steps i-k for one sample at a time to avoid media setting before pouring
   9. In a laminar hood add 0.05g Rifampicin and 238ul Tergitol to PDA flask and mix lightly. Avoid making bubbles in the media
   10. Add the each sample to a flask containing 50ml selective media
   11. Pour the sample/media solution into 5 sterile petridishes
   12. Parafilm the petridishes
   13. Incubate at 30˚C for 3 days
       * **Note**: Step D is required to reduce the concentration of the bleach solution to prevent damaging/killing the M. phaseolina.
2. Count total individual M. phaseolina colony forming units (CFUs) on all plates for a single value per sample