# Surface sterilization protocol for roots and leaves : first steps for the isolation of endophytic microorganisms

## Objective:

Surface-sterilisation of host plant samples is an integral component of fungal endophyte work, and is used to remove epiphytic microorganisms from the surfaces of plant tissues. The time required in each sterilant can be determined in a pilot study and usually varies depending on the host plant tissue, age, sensitivity and thickness (Fröhlich, Hyde & Petrini, 2000; Hyde & Soytong, 2008; Schulz & Boyle, 2005; Schulz et al., 1998), and must be sufficient to sterilise the surface but not destroy the tissue (Schulz & Boyle, 2005). Any fungi that are subsequently isolated from the plant samples are assumed to be endophytic, so it is crucial to have confidence in the surface-sterilisation process.

## Materials:

* Ethanol 75 %
* Ultra-Pure Water
* Bleach 9 %
* CTAB buffer 2 % (Cetrimonium bromide)
* Plastic container : 4 x 6 well plates.
* Scissors
* Tweezers
* Beakers
* 1 mL pipet
* 12,5 mL pipet

## Method (protocol adapted from XXX):

**Part 1** : Weight the sample (ecophysiology lab)

**Part 2** : Sterilization (Genetics’ lab)

The tools (tweezers and scissors) used for leaf/root handling have to be sterilized prior to each use, by dipping them into 75% alcohol and flaming over an electric bunsen burner.

Make sure the 4x6 well plates are sterilized prior to each use by placing them under UV radiation for 20 min.

Soak the samples in a series of solutions as followed:

1. Soak in 2 mL Ethanol 75 % for 30s
2. Rinse in 2 mL of ultra-pure water for 30s
3. Soak in 2 mL bleach 9 % for 30s
4. Rinse in 2 mL of ultra-pure water for 30s – repeat again for 30s
5. Soak in 2 mL of CTAB buffer 2 % for 30s : CTAB serves as an important surfactant in the DNA extraction buffer system to remove membrane lipids and promote cell lysis.
6. Place sample in 15 mL tube
7. Keep 2 mL of the CTAB bath for the negative control. The success of this surface sterilization method is confirmed by the absence of microbial sequences found in this last step.
8. Store samples at -20°C
9. Throw in the sink the solutions. Clean plastic containers with ultra-pure water.