GROW
NEXT GEN

Standard Laboratory Operating Procedure #100
Effectiveness of Alcohol for Surface Sterilization of Leaf Tissues

Laboratory: Biotechnology **Location**: Lab Station
SOP prepared by: R. Sanders and B. Wolf **Last Revision:** 6 January 2014

General: The goal of this experiment is to examine the effectiveness of surface sterilizing leaf tissues with alcohol prior to in vitro collecting plant tissue cultures. In vitro collecting or IVC is a method for starting tissue cultures in the field for conducting field research or collecting endangered plant species.

Safety: Safety Glasses
Caution: The 70% alcohol is flammable and these procedures should not be done near open flame.

Materials:
70% Isopropyl Alcohol
Small Container for Alcohol
Cheesecloth or Sterile Gauze Pad
2 Nutrient Agar Plates
Plant with young growing leaves

Procedure:

1. Fill small container with 50 mL of 70% Isopropyl alcohol and add a piece of cheesecloth to the liquid.
2. Label plate 1: Control (Before Sterilizing).
3. Label plate 2: Experimental (After Sterilizing)
4. Remove the leaf and as much of the petiole (leaf stalk) as possible from the plant.
5. Hold the leaf by the petiole in one hand and lift the lid of plate 1 with the other.
6. For 2-3 seconds, wipe the surface of the leaf over the surface of nutrient agar taking care not to touch the medium with your fingers. Immediately after swabbing replace the lid on plate.
7. Continue to hold the leaf by the petiole in one hand and pick up the cheesecloth soaked in 70% alcohol in the other hand.
8. Wipe the entire surface of the leaf with the alcohol soaked cheesecloth for 2-3 seconds, taking care not to touch the leaf to anything that is not sterile.
9. Still holding the leaf by the petiole in one hand, lift the lid of plate 2 with the other. For 2-3 seconds, wipe the surface of the leaf over the surface of nutrient agar taking care not to touch the medium with your fingers. Immediately after swabbing replace the lid on plate.
10. Use parafilm to seal the edges of each plate or if parafilm is not available then tape sides closed. Place the plates upside down on shelf of incubator at 37 degrees C for 48 hours. If incubator is unavailable, incubate plates upside down at room temperature for 72 hours.
11. Record number of bacterial colonies that grew on each plate in table data of lab notebook.