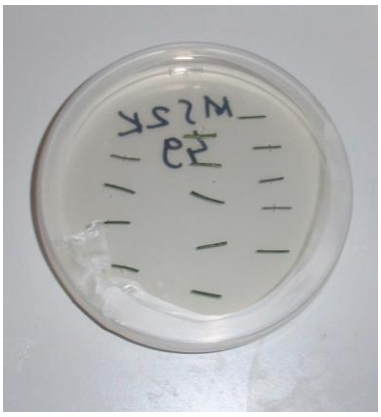
Detection of grass endophytic infection by placing plants scraps on medium in Petri dish

You will need:



- 75% EtOH

- 4% NaOCl

- Petri dishes with medium PDA (potato dextrose agar)

- empty Petri dish

- laminar flow chamber with UV

- wash bottle with alcohol

- parafilm

- scalpel

- tweezers

- burner placed in the chamber - best if it can be very easy to operate - for example, using the pedal on the floor (to keep your hands free)

- paper towels

Pour NaOCl into the first dish, into the second pour the alcohol – to fill the containers up to about half of the volume. Clean the table in the chamber with alcohol, then run chamber – turn on a light, air flow and the UV – leave the UV light for about 15-30 minutes.

In the meantime you can deal with pre-treatment of plant material – 3 tillers should be taken from each plant. Basal part of a tiller you should cut off a little bit above the base (no more than about 5 mm). On the opposite end a bit larger fragment should be removed so as to have in a result about 4 cm length sample for the analysis.

After the lapse of a 15-30 minutes and turning the UV off, place the tillers for 30 seconds in the Petri dish with alcohol and then for about 3.5 min. in the second dish with NaOCl and again for 15 sec. in alcohol.

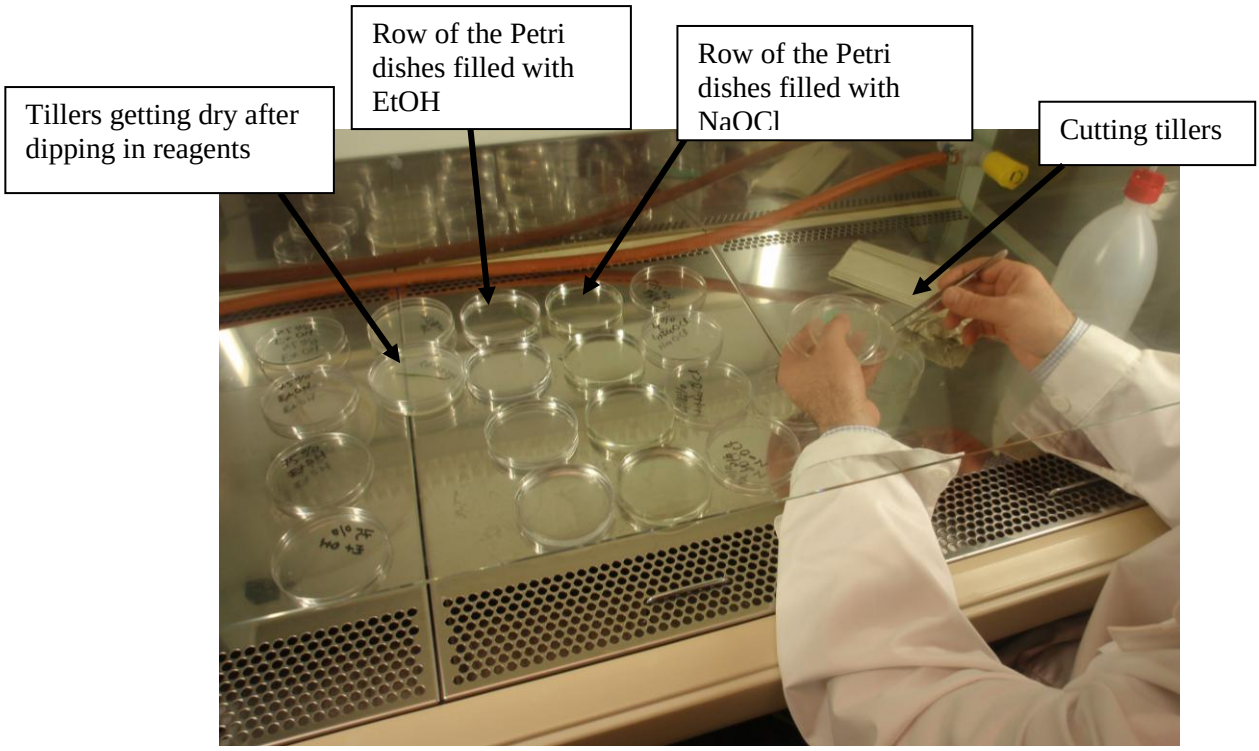
Then disinfect scalpel and tweezers with flame. After soaking plant material in alcohol and NaOCl place 3 tillers from one plant separately in the empty dish and cut each with a scalpel into 5 equal pieces. Be careful not to mix parts from different blades.

Then carefully with tweezers lay down pieces in a dish with the medium, but do not "push" them into medium just gently ‘parachute’ them above the agar surface. Fragments of one tiller should be placed in a one row - so in total on each plate there should be 3 rows, each consisting of 5 pieces. During placing the plants scraps on medium Petri dish should be only

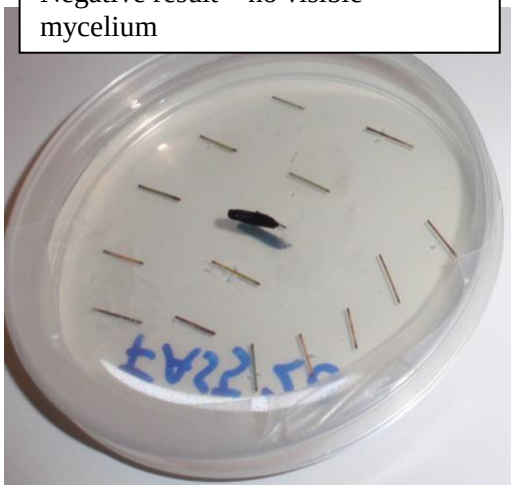
slightly not completely open. At the end you should stick prafilm over Petri dish and keep it at room temperature – after a week or two white mycelium becomes visible - usually growing out of the ends of grass pieces.

Before the preparation of the next shoots, from the next plant remember to decontaminate tweezers, scalpel and dish used for cutting tillers; the tools with flame and the plate with alcohol from wash bottle.

Preparation



Negative result – no visible mycelium



Positive result – white mycelium growing from both ends of scraps

Contamination – inconclusive result, analysis should be repeated