Isolation of endophytic fungi into pure culture

You will need:

- Petri dishes with tillers from which mycelium of endophyte grows out
- Petri dishes with medium PDA (potato dextrose agar)
- laminar flow chamber with UV
- wash bottle with alcohol
- parafilm
- dissecting needle

- 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

Clean the table in the chamber with alcohol, then run chamber – turn on a light, air flow and at the end UV – leave the UV light for about 15-30 minutes.

Then, using a needle you should collect from a plate, in which the white mycelium of endophyte is visible, small pieces (approximately 1 mm in diameter) of agar with the mycelium (usually three pieces from one dish), and place them into plate with fresh agar.

Then the plate should stuck be over with the parafilm. For several weeks – until the mycelium grow bigger – dish should be stored at room temperature, then it can be kept at a lower temperature (approximately 6 $^{\circ}$ C), which will slow down somewhat the rate of metabolism and the growth of fungus' mycelium and thus allows to store the sample for a relatively long period of time. Once a year mycelium can be transplanted to a new agar if you want to keep the sample.



Right after the endophyte transplantation on a new agar

The mycelia may also be placed on agar in falcon tubes, which may be particularly useful if one wishes to transport the sample - for example, sending it for the further analysis.



Isolates after few weeks

