Spore suspension and Spore counting

Introduction

The purpose of this protocol is to obtain the spores generated from a plate culture in agar.

Materials

- MilliQ (MQ) water
- Mira-cloth
- Autoclaved Funnels
- Falcon tubes (50 mL)
- Cell spreader
- Optical Microscope

Procedure

Spore suspension

1. Pour MQ water into the fungal culture plates.
2. Rub the plate with the spreader. Make sure the spores get into solution. Spores are hydrophobic and can spread easily into the surroundings.
3. After spores are in suspension, if more plates with the same spores are to be harvested, pour water and spores to the next plate. Repeat steps 1-2 for as many plates as you have.
4. Set a filter (funnel + mira-cloth) on a Falcon tube.
5. Pour MQ water + spores onto the mira-cloth and filter solution. Make sure all liquid goes through the filter.
6. Do a second filtration on a new Falcon tube and filter. Make sure all liquid goes through.

Spore counting

1. Make 1:100 dilution with the spore suspension (only 10 µL needed).
2. With a counting chamber under the optical microscope, put 5 µL of the dilution into the centre of the chamber. Count spores in one of the squared cells.
3. Calculate spore concentration.

A. niger Glycerol stock

Introduction

The purpose of this protocol is to prepare a stock of *A.niger*.

Materials

- An agar plate with *A. niger*
- 50% glycerol
- Appropriate media
- Eppendorf tube or storage tube
- Cryo-tube
- MilliQ water

Procedure

1. Pour 2.5 mL of 50% glycerol on the plate.
2. Use a cell spreader to harvest spores.
3. Transfer 2 mL to an Eppendorf tube or storage tube (for the -20°C stock).
4. Pour 750 µL of MilliQ water to a cryo-tube and transfer 500 µL from the spore suspension (for the -80°C stock).