

Storage of *Phytophthora infestans* in liquid Nitrogen

Storage of sporangia suspensions.

- Place leaflets of potato cultivar Bintje abaxial side up in a Ø 90-mm Petri plate on 1.5 % water agar (WA).
- Place little drops (10 µl) of tap water on the leaflets
- Transfer a small mycelial plug from an infected tuber slice or a Petri plate to the droplets.
- Place the Petri plates with the inoculated leaflets in a plastic tray covered with a transparent plastic bag.
- Incubate the inoculated leaflets for 1 week at 15°C at a light intensity of 12 Wm⁻² 16 hours a day.
- Rinse a fully diseased leaflet in a beaker with 6.5-ml 15% sterile Dimethylsulfoxid (DMSO) to collect sporangia. This has to be done in a flowcabinet.
- Transfer 1.5-ml sporangia suspension to nunc cryotube vials (1,8 ml) and close the vial. We store 3 vials per isolate.
- Put the vials within the hour in a polystyrene foam box at -80°C for 24 hours.
- Quickly transfer the vials into the liquid nitrogen.

Storage of mycelium plugs

- Grow mycelium of the isolate you want to store on a rye agar plate for 1-2 weeks. Incubate at 20°C.
- Transfer 5 plugs of fresh mycelium and agar (cut with a sterile instrument) to a nunc cryotube vials (1.8-ml). Work in a flowcabinet
- Add 1.5-ml 15% sterile Dimethylsulfoxid (DMSO) and close the vial.
- Within the hour put the vials in a polystyrene foam box at -80°C for 24 hours.
- Quickly transfer the vials into the liquid nitrogen.

For thawing: take the vials out of the liquid nitrogen and leave the vials at room temperature for slow thawing. Sporangia suspension can be put directly on potato slices. Plugs can be transferred to Rye agar or Pea agar plates.