

Long-term storage of *Verticillium* strains at -80°C

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1. Grow *Verticillium* strain on agar medium of choice, PDA works fine. Check whether conidia are formed. If there are only a few conidia, see comments below. Continue to the next step once colony diameter reaches $\frac{3}{4}$ of Petri dish diameter.
2. Prepare a glycerol solution: For 50 mL solution, mix 0.6 g Potato Dextrose Broth medium (Difco), 37.5 mL water and 12.5 mL glycerol.
3. Label top and side of 2 mL sterile screw-cap freezer vials with strain ID using black marker. Label 2 vials for each strain.
4. Add about 3 mL glycerol solution into each culture plate using a sterile pipet, and dislodge the conidia with a sterile glass rod or similar tool.
5. Transfer half of the conidia suspension into each freezer vial using a pipet with its tip cut off (use sterile scissors).
6. Place vials on their side in a -80°C freezer to prevent sedimentation of the conidia to the bottom of the vial. You may also use liquid nitrogen to quickly freeze them. Once frozen, transfer vials into a freezer box.
7. To grow the strains from glycerol stock, use a sterile wooden applicator or needle to transfer a small amount of the frozen spore suspension onto agar medium.
8. Promptly return vial to freezer. **NEVER LET VIAL THAW COMPLETELY.** Thawing significantly reduces their viability.

When cultures form few conidia:

There should be clouds of conidia in wet mounts of sporulating cultures. If only a few conidia are visible, the viability of the freezer stock will be reduced. To concentrate conidia, culture the strain on several Petri plates, and pour the conidial suspension from the first plate into the next plate, dislodge the conidia, and so on. Check final conidia concentration under the microscope.