

Long-term storage of *Zymoseptoria tritici* on silica gel

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Abstract

Preparation of *Zymoseptoria tritici* long-term storage on silica gel. Rodney Pettway 1992, ed. Petteri Karisto 2018.

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Protocol

Preparation of the silica gel

Step 1.

Always begin by baking (**dry** sterilization) a fresh batch of silica gel powder before preparing tubes. [Optional: Mix 1 part indicator silica gel with 9 parts silica gel (10% indicator gel by volume).] Place in shallow layer in baking tray or glass beaker and dry heat sterilize in oven at 190 C for 3-4 hours. [Transfer dried, sterilized silica gel to sterile glass beaker]. Cover the beaker tightly with foil, and allow to cool to room temperature for approx. 30 minutes.

Preparation of the tubes

Step 2.

Sterilize some 2 ml screw top vials in autoclave. Be sure these are absolutely dry before using them.

Fill tubes with silica gel

Step 3.

Add silica gel to within one centimeter of the bottom thread on the screw top vial. Use a funnel to add silica gel to vials. Label tubes.

Add spores in the tubes

Step 4.

Add 150 microliters of a fresh spore (or yeast cells) solution to the silica gel vials. Be sure to mix spores well before pipetting up sample. Disperse the spores over the silica gel by shaking the vial and rapping with your finger with the top loosely screwed down. After the spores are well-dispersed (max 15 sec), screw the top down as tight as possible, and transfer immediately to the ice bucket. Leave the vials in the ice bucket until all isolates have been transferred to silica gel tubes.

Store the tubes

Step 5.

Place isolates in a labelled storage box. [Optional additional protection: place the storage boxes in a container with approx. two cups of fresh silica gel in the bottom. Label outside of the container with description of isolates in the container.] Store the isolates in -80 degrees Celsius. Properly stored isolates can be safely stored for at least 10-15 years.

Using the isolates

Step 6.

Transfer spore clump from the tube on agar plate and spread on the plate to get fresh colonies. Check indicator crystals in tube after every time they are opened. If all indicator crystals indicate humidity, prepare a fresh long-term storage vial for this isolate.