Store preparation using Sigmacote coated tubes

Spores are prepared taking advantage of their high hydrophobicity

Amy Gladfelter Lab: Fall Rotation 2013

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Preparation of the Sigmacote Tubes

- Add 250µl of Sigmacote (toxic) to a glass test tube with screw cap.
- Distribute it all over the tubes, remove the rest and let dry over night at 37°C.
- Autoclave the tubes.
- The silicone is now covalently bound to the glass and tubes are ready to use.

Collecting the Spores

- Add 10 mL sterile H_2O (sddH_2O) to the tube and add the mycelium from one plate.
- Shake vigorously to destroy the mycelium.
- Let tubes incubate for 1 hour in a turning wheel at 37°C to give the spores time to stick to the wall.
- Discard the water and rinse the tube 3x 10 mL with pure water (sddH_2O) to remove residual pieces of mycelium.
- Add 10mL 0.1% Triton and shake and vortex vigorously to wash the spores down from the glass wall. Let sit for 10 minutes.
- Invert tube 1x to gentle mix/suspend spores and then pour transfer to a 15mL Falcon tube.
- Collect the spores by centrifugation; 3G for 4 to 5 minutes.
- Discard 0.1% Triton, leaving pellet in Falcon tube. Wash spores with 5mL spore buffer (0.03% Triton) and centrifuge at 3G for 4 minutes. Repeat wash.
- After 2 washes, take spores up in 500 µL spore buffer and 500 µL 50% glycerol and transfer to cryo tube. Freeze immediately.
    - The washing steps may be important to guarantee proper growth properties of the spores.
    - For time-lapse, make sure most of the Triton is removed or the spores will not germinate (e.g. dilute them with growth medium or let them grow in 50mL AFM before adding them in the time lapse slides.