Metsch farms protocol V.2

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This is a protocol to infect and maintain Daphnia with *Metschnikowia bicuspidata* for use in laboratory experiments.

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This is a protocol to infect and maintain Daphnia with *Metschnikowia bicuspidata* for use in laboratory experiments.

Metsch farms protocol

Written by Katherine Hunsberger
Additions made by Rebecca Bilich on 3/2/2021

Set up: start a farm with ~75 Standard animals and a dose of 250 sp/mL if spores are new (1-2 weeks old) or 500 sp/mL if spores are older (2 weeks-2 months)

Use a 1000 mL Erlenmeyer flask and add Daphnia from a beaker of 75 by running them down the side of the flask, then rinsing the inner sides of the beaker and flask to dislodge any stuck animals. Any 75

Standards will work but juveniles of intermediate age are most likely to get infected, however it is still a good idea to also include a variety of ages in the farm.

Add water to bring the final volume of the farm up to 900mL.

Select standards already infected with standard metsch to create the spore slurry. To infect at 250 spores/mL, use animals 2 weeks to a month old. To infect at 500 spores/mL, use animals 1 month to 2 months old. Younger spores may produce infections but also run the risk of killing the host too soon after exposure. Older spores are unlikely to produce infections.

Estimate the number of animals needed by supposing that each infected animal contains 30,000 metsch spores.

Grind the animals up in microcentrifuge tubes using an electric pestle from 60 seconds, making sure to make a homogenous mixture with no obvious Daphnia parts. Then remove the slurry to a small (5 mL) beaker. Then rinse each tube with about 0.5 mL of filtered lake water, vortex the tubes for a few seconds, and add this water to the slurry as well.

Mix the slurry well with a pipet for 60 seconds and then add 10 microliters to four wells in a Neubauer Hemocytometer. Do 4 cell counts, counting the entire grid, excluding metsch spores that do not appear viable (degraded or immature spores.) 20-30 spores on the grid is a good number to give an accurate count, but fewer spores than this on the grid is a sign of slurry that is very dilute and hard to accurately estimate the density of.

Choose 3 or more counts that are within 85% of each other, take the average of this number, and multiply it by 10,000 to get the concentration of the slurry in spores/mL.

Use the equation M1*V1=M2*V2 to find the volume of slurry to add to each farm. Add this amount with a pipet and then feed each farm only 10 mL of food (after adding the spores, to mix them around.) Tap the flask with a small amount of cetyl alcohol. Also feed only 10 mL on the next feeding day to encourage Daphnia to consume metsch spores. On subsequent feeding days feed 20 mL.

Maintenance: check farms every 10 days

- If there are a decent amount of infections and still uninfecteds, leave as is
- If there are lots of infecteds, but few uninfecteds, add more susceptible Stds
- If there are few infecteds, but many uninfecteds, add more Metsch
- If there are few infecteds & few uninfecteds, take down farm

Storage: store in fridge, but keep a few farms going at all times to maintain fresh Metsch