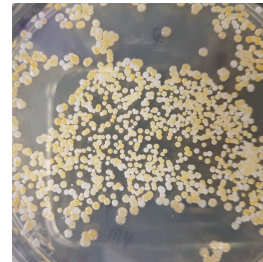


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Zoospore Isolation of *Aurantiochytrium limacinum*

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Mariana Rius¹

¹State University of New York at Stony Brook

Protist Research to Opti...

Collier Lab



Mariana Rius

Stony B

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol results in the isolation of single nucleated zoospores from a culture of *Aurantiochytrium limacinum*. The point is to recover single nucleus colonies rather than single cell colonies (which may contain varying nuclei - particularly following transformation).

Materials

Filtration Pump (or other way to pull a vacuum)

Filter holder (mftr#: Thermo Scientific Nalgene 300-4000)

5 um nylon filter (catalog#: R50SP04700)

15 ml culture tube

GPY media (+ zeocin 100 ug/ml)

- 0.5% Yeast Extract, 1% Peptone, 3% D+-Glucose, 1.8% instant ocean

GPY zeocin plate (+ zeocin 100 ug/ml + ampicilin 100 ug/ml)



Prepare Culture

- 1 Inoculate 500 ul GPY media (microcentrifuge tube) with toothpick scrape of a colony. If inoculating a zeocin resistant colony, add 5 ul zeocin (for 100 ug/ml). Vortex/ resuspend cells. Pipette 4.5 ml of GPY into a 15 ml glass culture tube. Add 500 ul inoculum. Incubate at 28 C, 170 rpm for 48 h, or until microscopy reveals the presence of zoospores.

Filter Culture

- 2 Filter culture using a 5 um nylon filter membrane, nalgene filter holder, and filtration pump. Transfer flowthrough to a microcentrifuge tube and centrifuge 5 min 11000 rpm.

Plate Zoospores

- 3 Resuspend cell pellet in 100 ul of $\frac{1}{2}$ Artificial Seawater (ASW) or media. Make a 10X dilution by adding 10 ul of cells to 90 ul of $\frac{1}{2}$ ASW or media. Spread onto 2% agar GPY (+ zeocin +amp) plate. Incubate at 28 C for 48 - 72 h until colonies appear.