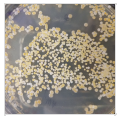

Zoospore Isolation of Aurantiochytrium limacinum

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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).

TAGS

isolation

zoospores

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

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 ½ Artificial Seawater (ASW) or media. Make a 10X dilution by adding 10 ul of cells to 90 ul of ½ ASW or media. Spread onto 2% agar GPY (+ zeocin +amp) plate. Incubate at 28 C for 48 - 72 h until colonies appear.