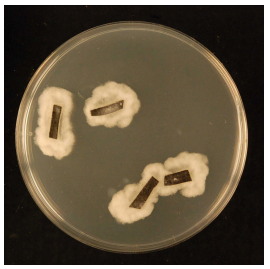
Nov 14, 2023

Storage & Revival of Oomycetes



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We use this protocol and it's working

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Abstract

Cryopreservation of oomycetes such as Phytophthora and Pythium species is essential for the long-term maintenance of these organisms. In this method we present an improved cryopreservation protocol for oomycete cultures, using carbon filter paper, to enhance their viability during liquid nitrogen storage with consistency. Our method benefits researchers working with these organisms by long-term, who do not have access to a programmable freezing equipment.

Guidelines

Here are some additional tips for storing and reviving chromists:

- Use young, actively growing cultures for storage.
- It is highly important to ensure hyphae does not get damaged during storage (steps 7-10)
- Ensure the cultured carbon filter strips are fully immersed in 10%-20% glycerol for 1-3 hours as some may display hydrophobicity.
- If the culture does not revive after the first attempt, try thawing another straw and using a different medium. Some species may prefer a solid medium such as Rye B agar, or a different broth such as V8. Addition of B-sitosterol (0.05g/L dissolved in small amount of dichloromethane) to rye broth may help with revival.
- If the culture still does not revive, store the culture in 20% glycerol.
- If liquid nitrogen is not available, this method should also work for long term storage of oomycetes at -80 °C

Materials

- Rye broth

Protocol

NAME

Rye Broth Recipe

CREATED BY

Diana Lee

Preview

- Carbon Filter Paper cut into strips

Equipment

Whatman® application specific filter, activated carbon loaded paper, Grade 72 NAME

Whatman BRAND

WHA1872-050 SKU

https://www.sigmaaldrich.com/NZ/en/product/aldrich/wha1872050 LINK

- Mr Frosty

Equipment

Mr. Frosty Freezing Container	NAME
Thermo Scientific	BRAND
5100-0001	SKU
https://www.thermofisher.com/order/catalog/product/5100-0001?cid=ANZ_LPX_CO_0SE_GAW_NT_mr-frosty-freezing-container_7012t000000YF4J&gclid=EAIaIQobChMI7frz_MvBggMVDNMWBR3XYQdBEAAYASAAEgK3kPD_BwE	LINK

- Glycerol solution (10%-20%)
- Petri dishes, non-vented
- Cryovials
- -80 °C Freezer
- Liquid Nitrogen Dewar

Troubleshooting

Safety warnings

Follow safety protocols required for handling of liquid nitrogen.

Preparation of Oomycetes for Storage

1 Inoculate a small agar plug of the oomycete culture into a thin film of rye broth in a petri dish.

2 Incubate at 20 °C until mycelium has grown about 4-5cm in diameter.

3 Tear the mycelium mat and place the small pieces onto carbon filter paper strips laid out on rye agar (or V8, or PDA).

4 Incubate at 20 °C until dense aerial mycelium is observed growing on the strips, usually in 3-7 days. 1w

5 Tear off the cultured carbon filter strips from the agar and place 4-5 strips into a petri dish containing a thin film of rye broth.

6 Leave the strips in the broth for 16-24 hours, or longer if the culture is slow growing. This process allows the hyphae to repair any damage caused by being torn off the agar. 1d

7 Rinse the strips in two changes of 10-20% glycerol to remove as much rye broth as possible. You can do this by gently swirling the strips in glycerol solution inside a petri dish.

8 Place the strips in a petri dish of fresh 10% glycerol solution and leave it sitting for 1-3 hours (at least 3 hours if the culture is slow growing) for the glycerol to be up taken into the cells.

9 Dab as much liquid off from the strips onto sterile filer paper laid out onto an empty petri dish.

10 Place the strip into a cryovial using sterile equipment and fill the vial with 10% glycerol, ensuring the entire filter strip is immersed.

11 Freeze at -80 °C overnight then place into liquid nitrogen storage. It is best to slowly freeze the culture inside something similar to a Mr. Frosty that has a cooling rate of -1 °C per minute., but not necessary.

Revival

2w

12 Immediately thaw the cryovial in water warmed to 30-40 °C

13 Place the culture strip into a petri dish containing rye broth.

14 Incubate at 20 °C for 7-14 days and watch for growth.

2w

15 Subculture the culture onto 10% V8 agar or PDA to check for contamination and morphology.