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Media for Fungal Culturing

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1 Works for me dx.doi.org/10.17504/protocols.io.bnuwmexe

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes the different media for fungal culturing.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

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Equipment for fungus culture work:

- burner
- ethanol bath for utensils
- ethanol spray
- petri dishes for dissection
- tape
- forceps

- gloves
- Kimwipes
- lighter
- media plates
- parafilm
- permanent lab marker
- scalpel
- scissors
- pipette and tips for melting & crushing
- Tween
- (loop for yeasts)

Beetle samples:

- vials with EtOH and labels for beetle vouchers

Preparing media plates

1. after the plates have cooled, mark each stack with a line of the appropriate color (easy when plates are stacked)
2. put them back in sleeves and tape those over
3. On the tape, write the
4. **media type**
5. **your name**
6. **due date**. The "due date" means that, if found in the fridge after this date, it can be discarded.
7. Wipe the hood completely with ethanol or bleach.
8. Store the new plates in the fridge.
9. In a week, check whether there are any contaminant colonies present. If a few are present, discard the contaminated plate(s). If many, discard the whole sleeve.

Nutrient media for abundant mycangial fungi

- Additional media formulations can be found in the media table in the isolations database. Please add new media formulations to the table in the database.PDA: 39g PDA dried media from BD-Difco, 1L water, 10ml of Streptomycin (10,000 I.U./ML) & Penicillin (10,000 MCG/ML) mixture. To our experience, this produces richer cultures of mycangial fungi than other media. It's also rather acidic and so prevents growth of many bacteria even without the antibiotics. Can add 5g of agar for harder media.
- YMEA: (4 g yeast extract, 10 g malt extract, 4 g dextrose, 15 g agar, 1L water), 10ml of Streptomycin (10,000 I.U./ML) & Penicillin (10,000 MCG/ML) mixture per 1L of media. Fungi grow slower than on PDA.
- MEA: same as YMEA, without the yeast extract.
- PYDA: 15g PDA, 10g Agar, 2g Yeast Extract, 1L Water.
- MYEA: 15g MEA, 10g Agar, 2g Yeast Extract, 1L Water.

Antibiotics

DO NOT AUTOCLAVE AGAR MEDIA WITH ANTIBIOTICS IN IT! Add when media has cooled down to touch.
Streptomycin/Penicillin: catalog #516106, supplied in powder. Mix into liquid media at a concentration of 100-200 ppm. For PDA, 100 mg/L is equivalent to 100ppm.
Add cycloheximide for Ophiostomatales, including Raffaelea (but not Ambrosiella). Kolarik & Hulcr (2008) used 0.1 mg/L cycloheximide to select for ophiostomatoid mycangial fungi, people use betweeen 0.1-0.5mb/L. We are using 0.5 mg/L cycloheximide in media.
Excessive growth of yeast (often on YMEA) can be prevented by slapping a chunk of agar on top of the inoculum.
All media containing antibiotics should be kept in darkness and cold temperature, to prevent degradation.

Ophiostoma select agar – OSA (aka CSMA)

- 34 g malt extract agar
- 4 g agar
- Bring to 1 L volume with deionized H2O

Autoclave
After autoclaving, at about 45 C add: (this is very warm but not painful to touch)

- 0.1 g cycloheximide
- 0.1 g streptomycin sulphate

Standard acidified potato-dextrose agar (APDA) with antibiotics:

-39 g potato dextrose agar (Difco Laboratories, Detroit, Mich.)

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-1 L deionized H2O
Autoclave
-1.25 mL of 20% lactic acid
-Streptomycin – 100 mg/L
-Chloramphenicol – 16 ug/ml -- .016 mg/L

Acidified weak potato-dextrose agar (AWPDA) with antibiotics:
-1.2 g potato-dextrose broth (Difco Laboratories)
-16 g Bacto-agar (Difco Laboratories)
-1 L deionized H2O
Autoclave
-0.3 mL of 20% lactic acid
-Streptomycin – 100 mg/L
-Chloramphenicol – 16 ug/ml -- .016 mg/L

MEA acidified with antibiotics:
-33.6 g MEA (Difco Laboratories, Detroit, Mich.)
-1 L deionized H2O
Autoclave
- 1.25 mL of 20% lactic acid
-Streptomycin – 100 mg/L
-Chloramphenicol – 16 ug/ml -- .016 mg/L

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