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Media and reagents for *Seminavis robusta* cultivation and experiments

In 2 collections

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Lev Tsypin[1], Aaron Turkewitz[2]

[1]California Institute of Technology; [2]University of Chicago

Protist Research to Opti...

Lev Tsypin
Stanford

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We use this protocol and it's working

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Abstract

This protocol describes the culturing conditions used to grow *S. robusta* in the lab for all experiments.

Guidelines

It matters whether you use NanoPure/MilliQ water or glass distilled water. In glass distilled water-based media, *S. robusta* tends to disadhere from the bottom of the culture flask and float more readily than in MilliQ-based media.

Materials

MATERIALS

- Bioactif artificial sea salt **Tropic Marin** **Catalog #**10304
- Guillard's (F/2) Marine Water Enrichment Solution **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G9903-500ML
- Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S6014
- Penicillin G sodium salt **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P3032-25MU
- Gentamycin sulfate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**345814-1GM-M
- Ampicillin sodium salt **Fisher Scientific** **Catalog #**BP1760-25
- Streptomycin sulfate **MP Biomedicals** **Catalog #**100556
- Agar **Fisher Scientific** **Catalog #**BP1423-500
- 175 cm2 flasks with sealing caps **Thermo Scientific** **Catalog #**156502
- 25 cm2 flasks with sealing caps **USA Scientific** **Catalog #**CC7682-4325
- Stericup **Merck Millipore (EMD Millipore)** **Catalog #**SCGPT05RE
- Cell Scraper **Fisher Scientific** **Catalog #**08-100-241
- D-sorbitol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M4125
- Mannitol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S1876

Preparation of F/2 medium

1 Dissolve 34.5 g Tropic-Marin Bioactif (TMB) in 1 L MilliQ water

2 Add 80 mg sodium bicarbonate

3 Autoclave

4 After cooling, add 20 mL 50x Guillard's supplement

5 Filter using Stericup into pre-sterilized bottles

6 Store at 4C in the dark

Maintenance antibiotics (200x stock)

7 Dissolve 1 g penicillin G sodium salt, 1 g ampicillin sodium salt, 0.2 g streptomycin sulfate, and 0.1 g gentamycin sulfate in a final volume of 20 mL F/2 medium.

8 Aliquot and store at -20 C.

Agar plates

9 Dissolve 17.25 g TMB in 1 L MilliQ

10 Add 80 mg sodium bicarbonate

11 Add 0 µL 15 g agar , dissolve while stirring and heating (may require boiling)

12 Autoclave

13 After cooling below 60 C, add 20 mL 50x Guillard's supplement , as well as selective antibiotics, if desired.

14 Pour approx. 19 mL per plate

15 Store in the dark at 4 C

Electroporation buffers

16 [M] 1 Molarity (M) sorbitol in MilliQ, [M] 0.77 Molarity (M) mannitol in MilliQ, or [M] 0.77 Molarity (M) mannitol with [M] 8 % volume F/2 in MilliQ.