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Cultivation protocol for anaerobic ciliates

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1 Works for me dx.doi.org/10.17504/protocols.io.85why7e

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MATERIALS

NAME	CATALOG #	VENDOR
Potassium Chloride		
Magnesium chloride hexahydrate	View	Sigma Aldrich
500g Calcium Chloride,Dihydrate (CaCl2.2H2O)	RC-030	G-Biosciences
NaCl	53014	Sigma Aldrich
Disodium phosphate	S7907	Sigma Aldrich
cerophylle	470300-680	Ward's Natural Science Establishment, Inc.

Sample isolation

1 Cultivation protocol is followed by methods described in Rotterová et al. 2018 and Bourland et al. 2017.

Rotterová J, Bourland W, Čepička I (2018). Tropidoatractidae fam. nov., a Deep Branching Lineage of Metopida (Armophorea, Ciliophora) Found in Diverse Habitats and Possessing Prokaryotic Symbionts.. Protist.
https://doi.org/10.1016/j.protis.2018.04.003

Bourland W, Rotterova J, Čepička I (2017). Redescription and molecular phylogeny of the type species for two main metopid genera, Metopus es (Müller, 1776) Lauterborn, 1916 and Brachonella contorta (Levander, 1894) Jankowski, 1964 (Metopida, Ciliophora), based on broad geographic sampling.. European journal of protistology.
https://doi.org/10.1016/j.ejop.2016.11.002

Isolate 10 ml of targeted sample from environments of interest (e.g., freshwater sulphidic sediment).

Media preparation

2 Prepare **ATCC medium: 802** (Sonneborn's Paramecium medium) for freshwater ciliates or **ATCC Medium: 1525** (Seawater Sonneborn's Paramecium Medium) for marine ciliates. Preparations for both below:

2.1 **Instructions for 500 ml of ATCC #802 Freshwater Cereal Grass Media (**Sonneborn's Paramecium medium) **preparation:**

- 500 ml dH2O
- 1.25 g cerophylle
- Boil for 5 minutes
- Filter through filter papers
- Add 0.25 g Na_2HPO_4
- Autoclave at 121 °C

Add cerophyll to distilled water and boil for 5 minutes. Add 100 ml distilled water to compensate for evaporation. Filter through Whatman #1 filter paper and add 0.5 g Na_2HPO_4. Autoclave for 15 minutes at 121 °C.

2.2 **Instructions for 1 liter of ATCC #1525 Seawater Cereal Grass Media** (Seawater Sonneborn's Paramecium Medium) **preparation:**

4 parts:

Cereal Grass Medium:
500ml bottle: Cerophylle

- 500 ml dH2O
- 2,5 g of cerophylle
- Boil for 5 minutes
- (it is twice as concentrated as the usual cereal grass media for freshwater cultures, + no Na_2HPO_4 included)
- Filter through filter papers

Artificial seawater protocol:
1 L bottle: SWA

- 300 ml dH2O
- 24.72 g NaCl
- 0.67 g KCl
- 1.36 g $CaCl_2$.2H2O
- 4.66 g $MgCl_2$.6H2O

100ml bottle: SWB

- 100 ml dH2O
- 6.29 g $MgSO_4$.7H2O

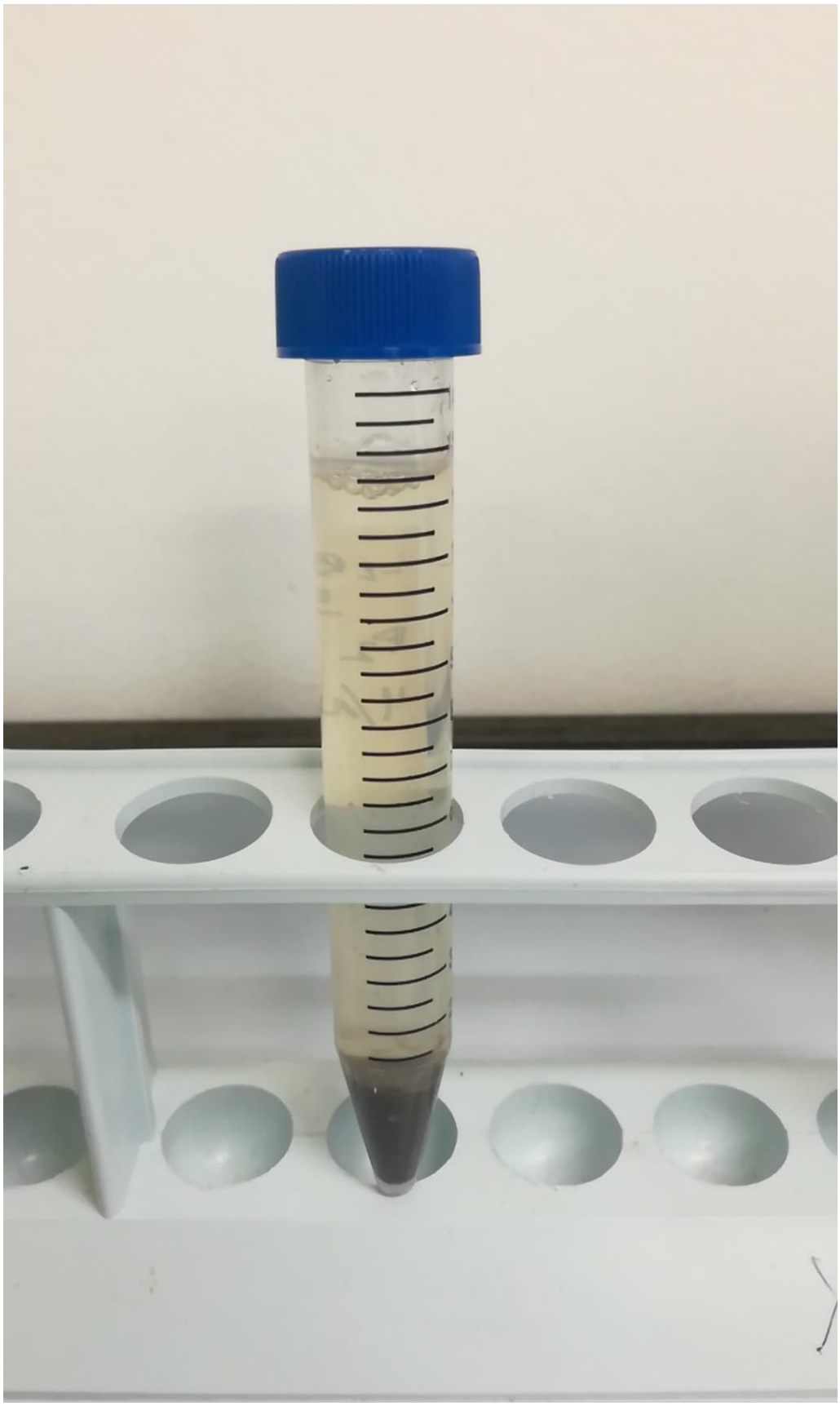
100ml bottle: SWC

- 100 ml dH2O
- 0.18 g $NaHCO_3$

Autoclave separately at 121 °C, mix after cooling down.

Cultivation establishment

3 Add each 1 ml of the sediment four times into a 15 ml falcon tube with 10 ml of the designated media.



Cultivation maintanance

4 Reinoculate 1 ml of each sedimented culture into a new 15 ml falcon tube with 10 ml of the designated media every two weeks.

Green organic matter from the cereal grass media will be gradually generated over time and the culture will lose anorganic particles, such as sand grains and soil, present in the original sample.

If you have any questions, please contact me here or at johana.rotterova@natur.cuni.cz.