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Collection and processing of intertidal seagrass tissues for bacterial and fungal culturing

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.butrnwm6

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ABSTRACT

This protocol details collection and processing of intertidal seagrass tissues for bacterial and fungal culturing.

EXTERNAL LINK

https://journals.plos.org/plosone/article/comments?id=10.1371/journal.pone.0236135

ATTACHMENTS

Collection and processing of intertidal seagrass tissues for bacterial and fungal culturing.docx

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PROTOCOL CITATION

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MATERIALS TEXT

Materials for field collections:

- PVC pipe (2.375 inch diameter)
- 1L Nalgene bottles
- Coring device
- Ziplock bags

Materials for sample processing and inoculation on culture media:

- Flame sterilized scissors
- **1.5 mL** centrifuge tubes
- **1 mL** of autoclaved nanopure water
- Flame sterilized tweezers
- **1 mL** 0.5% NaOCl
- 95% EtOH
- **500 µl** 95% ethanol
- **500 µl** 70% ethanol

Field collections 1d 4h

1 Put on gloves.

2 Walk to a location that is submerged (~0.5 m).

3 Using a coring device, take a sample of an individual seagrass plant and its associated sediment.

3.1 Insert a clear PVC pipe with a **2.375 Inch** diameter that has been modified such that one end of the pipe is cut at an angle to make insertion into the sediment easier.

3.2 Once inserted, Cap the PVC pipe, pull the core up and then cap the bottom of the core.

4 Place core in dark box **On ice** until transport back to the lab.

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5 Replace gloves and repeat to obtain multiple cores.

6 Collect nearby seawater in **1 L** Nalgene bottles (or similar) as needed for media recipes.

7 Using new gloves, carefully pull up additional seagrass plants as needed for media recipes and/or inoculation, place into sterile ziplock bags and then put **On ice** .

8 Once back at the lab, place cores, bulk tissues and seawater into **4 °C** fridge or cold room and use as needed within 1d 4h **04:00:00** - **24:00:00** of collection.

Sample processing and inoculation on culture media 15m 35s

9 30s

Plant tissues (leaf, root, rhizome, matte).
Before starting, prepare plates with your favorite culture media (e.g. see Ettinger & Eisen 2020 for possible media choices previously used for seagrass-associated work)

Rinse the tissue with autoclaved nanopure water to remove loosely associated sediment for ~ **00:00:30** .

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10 Cut ~ **1 cm** pieces of tissue using flame sterilized scissors

11 Place a subset of these tissue segments directly on plates using flame sterilized tweezers (1–3 segments/plate).

12 Take another subset of tissue segments and place these segments into **1.5 mL** centrifuge tubes with **1 mL** of autoclaved nanopure water.

13 

30s

Vortex the **1.5 mL** centrifuge tubes for ~ **00:00:30** .

14 For a subset of segments, homogenize using a sterile pestel, while for another subset leave the segments intact.

15 Directly plate intact rinsed tissue segments on media using flame sterilized tweezers (1–3 segments/plate).

16 

Pipette **350 µl** of wash liquid from rinsed segments or of homogenized smashed tissue directly on plates.

As needed, you can also pipette 1:10 and 1:100 dilutions of wash liquid on plates.

17

If of interest, one can try to remove epiphytes from tissues through several wash steps before plating.

One protocol is to bleach tissues prior to plating, for example:

17.1 First, immerse segments for **00:05:00** in **1 mL** 0.5% NaOCl (~10% bleach).

5m

17.2 Then immerse in **1 mL** of 95% EtOH for **00:01:00** .

1m

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17.3 Next immerse in **1 mL** autoclaved nanopure water for **00:03:00** . 3m

17.4 Finally directly plate intact bleached tissue segments on media using flame sterilized tweezers (1–3 segments / plate).

18 Another, slightly longer protocol to surface clean tissues, involves:

18.1 First, immerse segments in **500 µl** 95% ethanol for ~ **00:00:05** . 5s

18.2 Then, immerse in **500 µl** 0.5% NaOCl (~10% bleach) for **00:02:00** . 2m

18.3 Next, immerse in **500 µl** 70% ethanol for **00:02:00** . 2m

18.4  1m

Then rinse segments with autoclaved nanopure water for 0w 0d 0h 1m 0s **00:01:00**

18.5 Finally, directly plate intact surface cleaned tissue segments on media using flame sterilized tweezers (1–3 segments /plate).

Sediment 15m 35s

19

Place sediment into **1.5 mL** centrifuge tubes.

20 

Add **1 mL** of autoclaved nanopure water to tubes.

21  30s

Vortex tubes for ~ **00:00:30** .

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22

Pipette **350 µl** of sediment suspension directly onto plates.

As needed, you can also pipette 1:10 and 1:100 dilutions of sediment suspension on plates.

Seawater 15m 35s

23

Pipette **350 µl** of seawater directly onto plates.

As needed, you can also pipette 1:10 and 1:100 dilutions of seawater on plates.

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