Extraction of surface-community DNA from Ulva sp.

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Abstract

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Protocol

Step 1.

Extraction of surface-community DNA from *Ulva sp*

Step 2.

This method performs a direct lysis of the microbial community on the algal surface followed by an organic extraction and precipitation of the microbial DNA. *Ulva* plants do get damaged during the treatment, however eukaryotic microbes might be lysed. Therefore microscopic check of the algal surface before treatment and removal of basal parts (old and most likely covered by secondary colonisers) is necessary.

Step 3.

Materials:

- plastic bag/ container
- washing bowl/ container
- ice bucket

  - sterile knife/ surgical blade
  - sterile forceps
  - 1 litre of autoclaved calcium- and magnesium-free sea water (CMFSW)
    - recipe:
      - 25 g NaCl
      - 8 g KCl
      - 1 g Na2SO4
      - 04 g NaHCO3
      - distilled water to 1 litre
    - live stain (1:500 diluted in CMFSW)
    - tissue
    - balance
    - petri dish

- sterile 50mL Falcon tubes
- 3M Multi Enzyme Cleaner (sterile-filtered)
- large pipette tips (5ml) and pipettor
- sterile SS34 tube
- 95 % ethanol
- 70% ethanol
- 3M Sodium acetate (pH 5.2)
- TE-buffer
- Sterile water
- 2 ml microcentrifuge tubes

Step 4.

Method:

1. Collect several *Ulva sp.* plants by hand from intertidal pool and transfer into a plastic bag/ container with sea water (from the same site)
2. Transfer into the laboratory asap
3. Transfer plants into sterile washing bowl/ container with a bit of CMFSW and cut off the buttom part (2-3 cm) of the plant with a sterile knife and forceps
4. Chop the plant into piece of roughly 2-4 cm length/ width
5. Wash plant pieces 3 times for 1-2 min with CMFSW; make sure smaller bits and non-plant material get wash away
6. Take a couple of random pieces and perform a microscopic observation on it:
    1. place *Ulva* piece onto glass slide
    2. cover *Ulva* piece with appr. 10 µl of live stain
    3. place on cover slip and drain excessive liquid with a tissue
    4. observe under fluorescent microscope
    5. describe and note surface coverage of cells
7. Using a balance weigh out 12 g of plant wet-weight (drip dry) into a petri dish
8. Transfer 12 g of plant into a sterile 50 ml Falcon tube with 30 ml of CMFSW and 300 µl of 3M Multi Enzyme Cleaner; shake gently to make sure the plant surface is covered with the CMFSW/ cleaner mix
9. Incubate at room temperature
10. Perform microscopy after 30 min (see point 6)
11. If surface shows little cells left or is cleared, then go to point 12. Otherwise continue incubation (total 1 hour or 2 hours), until most cells on the plant surface are remove
12. Transfer liquid into 1 or 2 new 50ml Falcon tubes (file tubes not more than 20 ml); make sure no plant material is being transferred, optional the liquid can be filtered through a sterile 125 µm sieve of a 0.8 µm filter
13. Add an equal volume of phenol/ chloroform/ isoamylalcohol (25:24:1) to the supernatant and shake gently but thoroughly
14. Centrifuge for 5 min at 2000 x g
15. Transfer aqueous phase into a SS34 tubes
16. Add 1/10 volume of 3M sodium acetate and 3 volumes of 95% ethanol
17. Shake gently but thoroughly; note: a white precipitate will become visible

18. Incubate at –20°C for at least 2 hours
19. Centrifuge for 30 min at 20 000 x g
20. Remove supernatant
21. Add 10 ml of 70% ethanol to pellet
22. Centrifuge for 5 min at 20 000 x g
23. Remove supernatant and air-dry pellet
24. Resuspend pellet in 0.5 ml sterile water and transfer into 2 ml microcentrifuge tubeAdd 1/10 volume of 3M sodium acetate and 3 volumes of 95% ethanol
25. Shake gently but thoroughly; note: a white precipitate will become visible
26. Incubate at –20°C for at least 2 hours
27. Centrifuge for 30 min at 20 000 x g
28. Remove supernatant
29. Add 1 ml of 70% ethanol to pellet
30. Centrifuge for 5 min at 20 000 x g
31. Remove supernatant and air-dry pellet
32. Resuspend pellet into 50-100µl TE-buffer
33. Check appr. 1µl on agarose gel

Step 5.

Comments:

- Yield should be around 20µg from 12 g of *Ulva* material
- Some contaminants co-purify with the DNA and form a strong, white precipitate during the EtOH ppt steps; there might be a need to filter this precipitate out at one stage
- the cleaner incubation appears to be equally efficient when done o/n (>15 hours) at 14°C
- At this stage DNA is sheared, but more careful handling might avoid this problem
- 16S and 18S rDNA PCR has indicated a ratio of at least 1:100 for 18S versus 16S
- Semi-quantitative/ comparative 16S/18S rDNA PCR should be performed as part of the quality-check