Isolation of axenic Symbiodinium cultures

Tingting Xiang

Abstract

Citation: Tingting Xiang Isolation of axenic Symbiodinium cultures. **protocols.io** dx.doi.org/10.17504/protocols.io.qxzdxp6
Published: 23 Jul 2018

Guidelines

Before start

Before starting, make 10X sterile Daigo solution in artificial seawater (ASW):
Dissolve 2.56 g Daigo powder in 1L ASW and filter sterilize through a 0.22 µm filter.

Materials

- 50g Streptomycin Sulphate USP Grade RC-197 by G-Biosciences
- Agar-Agar by Contributed by users
- Ampicillin, sodium salt USP AB0028.SIZE.25g by Bio Basic Inc.
- Kanamycin sulfate KB0286.SIZE.100g by Bio Basic Inc.
- Homogenizer PowerGen 125 by Fisher Scientific
- DAPI D1306 by Thermo Fisher Scientific
- 4% Paraformaldehyde (PFA) Solution in PBS AR1068 by Boster Bio

✓ CORAL PRO SALT R_99344 by Contributed by users

✓ DAIGOS IMK MEDIUM FOR MARINE MICROALGAE 398-01333 by Contributed by users

Protocol

Purifiy algae from anemone host

Step 1.

Several anemones were disrupted using a glass homogenizer so as to leave the algal cells largely intact.

Purifiy algae from anemone host

Step 2.

The algal cells were pelleted by centrifugation at 1,000g and then washed by resuspension and repelleting the cells several times in filter-sterilized ASW containing 50µg mL^{-1} kanamycin,100µg mL^{-1} ampicillin, and 50µg mL^{-1} streptomycinantibiotics KAS (Kan, Amp and Strep)

Plating algal cells on solid medium

Step 3.

Prepare solid IMK medium: add 1g Agar into 90 mL ASW, and autoclave at 122ºC for 30 minutes.

Plating algal cells on solid medium

Step 4.

After autoclave, let it cool down to around 65 °C, add sterile 10X Daigo's IMK Medium stock solution as well as KAS antibiotic cocktail: 50µg mL^{-1} kanamycin,100µg mL^{-1} ampicillin, and 50µg mL^{-1} streptomycin.

Plating algal cells on solid medium

Step 5.

The algae in liquid solution were then spread onto solid IMK + KAS medium.

NOTES

1. To be able to have single colonies for later pick up or purification, plating different amount of cells are recommended.
2. Wrap plates in parafilm to prevent evaporation/drying of the medium.
3. Expected amount of time needed for visible colony growth is around 30 days, although some stains may take more or less time.

Plating algal cells on solid medium

Step 6.

Prepare a flame-sterilized inoculation loop, allowed to cool to room temperature for use;

Plating algal cells on solid medium

Step 7.

Then individual colonies were picked with the inoculation loop under microscope and streaked onto solid IMK + KAS medium.

Plating algal cells on solid medium

Step 8.

Repeat step 8 procedure for several times until a clean single colony was formed. Those are potentially axenic colnal colonies.

Verify clonal and axenic Symbiodinium culture

Step 9.

Identify if the colonies are bacteria-free by transfering them to rich solid medium Marine Broth (MB). If there is bacterial or other contaminatin, they will grow on MB plate.

Verify clonal and axenic Symbiodinium culture

Step 10.

If there is no contamination on MB plate, further verify by PCR using specific bacterial 16s primers. Extract genomic DNA from the colony/cells grown on the plate.

PCR reactions contains 0.3 M of each primer in 2X GoTaq Master Mix (Promega, Madison, WI, USA) and were performed
by incubating at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 50°C for 1 min, and 72°C for 1.5 min, with a final incubation at 72°C for 5 min.

NOTES

16S bacterial primers:
first set:
63F: 5′-CAGGCCTAACACATGCAAGTC-3′
1542R: 5′-AAGGAGGTGATCCAGCCGCA-3′

second set:
27F: 5′-AGAGTTTGATCCTGGCTCAG-3′
1492R: 5′-GGTTACCTTGTTACGACTT-3′

Verify clonal and axenic Symbiodinium culture

Step 11.

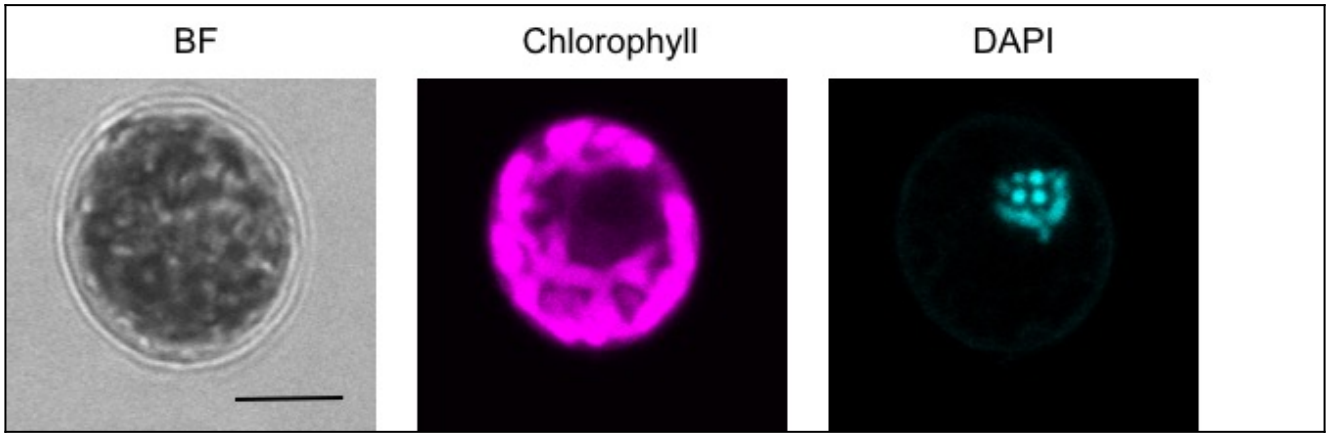
Further verify the axenic culture by imaging the cells under the microscope.
(1) Fix the cells by using 4% PFA for more than one hour in 4ºC;
(2) Wash the cells using PBS buffer for 6 times;
(3) Stain the cells with DAPI;
(4) Examine the cells by bright-field and fluorescence microscopy. If the culture is axenic, bacteria would

not be observed in the culture medium or associated with the Symbiodinium cells.

NOTES

Here is an example image of Symbiodinium cell from an axenic culture stained with DAPI.

Scale bar: 3 µm



Verify clonal and axenic Symbiodinium culture

Step 12.

To identify the Symbidonium, PCR using 18S and Chloroplast Cp23S sequences to assign the clades.

Detailed protocol can be found in the following link.

NOTES

Primer seqeunces:

18S-F: 5′-GGTTGATCCTGCCAGTAGTCATATGCTTG-3′
18S-R: 5′-GATCCTTCCGCAGGTTCACCTACGGAAACC-3′

Cp23S-F: 5′-GACGGCTGTAACTATAACGG-3′
Cp23S-R: 5′-CCATCGTATTGAACCCAGC-3′