Glycan Masking and Colonization Assay

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Abstract

Use this protocol to mask *Symbiodinium* cell surface glycans prior to colonizing aposymbiotic *Exaiptasia* polyps, then measure colonization dynamics with epifluorescent microscopy.

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Guidelines

The general strategy is to expose aposymbiotic anemone polyps to glycan-masked symbiont cells for two days, then remove the cells and allow one extra day of proliferation. Animals are fixed on the third day post exposure then symbiont density is determined through fluorescent microscopy.

Before start

Several variables affect symbiont uptake rate, such as how long the anemones are starved, how large they are, temperature, light, lectin concentration, and the density of symbionts used during exposure. Even keeping these constant, expect high variability during early uptake—try to use at least 5 polyps per treatment if possible. Here we present our usual approach, but adjustments may be necessary depending on the experiment.

Materials

- Filtered and autoclaved seawater (FSW; artificial seawater also works) by Contributed by users
- Aposymbiotic anemone polyps (~0.5 mm oral disc diameter; maintained in the dark at room temperature and starved for one day prior to the experiment) by Contributed by users
- Plastic flat-bottomed 24-well plates by Contributed by users
- Symbiodinium cultures of interest in F/2 media by Contributed by users
- Filtered Artemia homogenate by Contributed by users
- Lectins of interest individually resuspended in ASW (100 µg · mL-1) by Contributed by users
- Incubator set to 26 °C on a 12:12 L:D photoperiod at 15 µmol quanta · m-2 · s-1 by Contributed by users

✓ Relaxing agent: 0.376 M MgCl2 in ASW by Contributed by users

✓ Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS) by Contributed by users

✓ Glycerol mountant (90% glycerol in PBS) by Contributed by users

Protocol

Preparation

Step 1.

1 d prior to the experiment, place aposymbiotic anemones (0.5 mm oral disc diameter) in a 24-well plastic plate (1 polyp per well in 1 mL FSW). Seal the plates with Parafilm and maintain in darkness at room temperature, allowing the polyps to attach overnight.

Preparation

Step 2.

Either the day of the experiment or 1 d prior, prepare Symbiodinium from cultures at 1e6 cells · mL-1 media (total volume of each subculture = 1 mL for every polyp in a given treatment). For easy centrifugation, maintain subcultures in 15 mL falcon tubes (split into multiple tubes if necessary).

Glycan Masking

Step 3.

2 h prior to the experiment, mask the Symbiodinium cell surface glycans with lectins by centrifuging the subcultures at 1500 RCF for 5 min, pouring off the supernatant, and resuspending in 100 µg · mL-1 lectin (total volume = 0.33 mL lectin per 1e6 Symbiodinium cells). Treatment should last 2 h with gentle vortexing every 20 min. Upon completion, centrifuge the cultures as before and resuspend in FSW to a final concentration of 1e6 cells · mL-1. Repeat this wash at least once more.

Colonization

Step 4.

For the colonization experiment, remove the FSW from the anemone wells and replace with 1 mL treated symbiont cells at 1e6 cells · mL-1. Add 10 µL of Artemia just above each polyp to stimulate a feeding response and increase uptake. Seal the plates with Parafilm and place them in the incubator (26 °C on a 12:12 L:D photoperiod at 15 µmol quanta · m-2 · s-1).

Colonization

Step 5.

After 24 h (1 d post exposure), add an additional 1 mL FSW to each well, seal the plate, and return it to the incubator. This step dilutes the remaining symbionts but also reoxygenates the water to prevent anemone death.

Colonization

Step 6.

After another 24 h (2 d post exposure), move the anemones to a new plate with fresh FSW (1 mL in

each well). While moving the anemones, try to remove any accumulated Symbiodinium from the polyp surface, particularly in the sticky ring around the outer polyp wall, which is easiest to grab using fine forceps. Seal the plate and return it to the incubator.

Colonization

Step 7.

After another 24 h (3 d post exposure), replace the FSW with relaxing solution (1mL 0.376 M MgCl2 in FSW), allow 10 min for polyps to relax, then transfer them to a small volume of paraformaldehyde fixative at room temperature (30 µL of 4% paraformaldehyde in PBS in a 0.5 mL tube). Just make sure the volume is sufficient to cover the entire polyp. After 1 h, replace the fixative with PBS and store the polyps at 20 °C overnight.

Microscopy

Step 8.

To determine symbiont density via microscopy, use small dissection scissors to separate the polyp body from “head” (the oral disc and tentacles). Mount the “head” on a slide with glycerol mountant (90% glycerol in PBS), being careful to spread the oral disc and tentacles flat with as little overlap as possible.

Microscopy

Step 9.

Using an epifluorescent scope, capture separate .jpg images of the red channel (symbiont chlorophyll fluorescence) and green channel (host GFP fluorescence) under a 5x objective.

Microscopy

Step 10.

Using ImageJ software, run the thee provided macros to a. mask out the polyp mouth area, b. count the number of symbiont cells, and c. calculate the host tissue area. Divide cell counts by their respective host tissue areas to calculate symbiont cell density for each polyp.

Warnings

Standard $MgCl_2$ precautions during relaxing (slightly toxic; use PPE).

Standard paraformaldehyde precautions during fixing (highly toxic, flammable; use PPE, fume hood, proper waste disposal).