Colonization of aposymbiotic Aiptasia with Symbiodinium

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Abstract

This protocol describes briefly how to perform colonization of aposymbiotic Aiptasia with *Symbiodinium* cultures.

The method is based on the one described in Xiang et al., 2013.

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Guidelines

Be careful while working with *Symbiodinium* cultures to not introduce any contaminations which might overgrow the slow-growing algae. Best practice is to perform all steps involving open cultures under sterile conditions in a clean bench.
The medium of the cultures can be IMK or IMK with casein hydrolysate, if you want to assess the colonization rates of different host or algae strains make sure to use the same medium for all cultures.
A general consideration: The smaller the tank with the anemones, the less *Symbiodinium* culture is needed.

Before start

The colonizationworks most efficiently with *Symbiodinium* cultures not older than 2 months.

Fluorescence filters:
Every wavelength between 400 and 550 nm efficiently axcites the algal chlorophyll.
The chlorophyll fluorescence emission peaks between 650 and 720 nm.
This means that most RFP filter sets with a wide bandpass or longpass emission filter at above 600 nm will work well.
GFP longpass filter sets will work as well but only in combination with an RGB camera to distinguish red algal fluorescence and green autofluorescence of the anemones themselves.
These numbers are only rough estimations based on a few algal strains. For purchasing new filter sets it will be still best to test them before buying.

Materials

✓ Fluorescence stereomicroscope by
Contributed by users

Protocol

Preparation of the aposymbiotic anemones

Step 1.

A month before infection place the anemones in a clear container and 12 h light per day (20–40 µmol photons of photosynthetically active radiation/m^2/s) .

If any algae are present in the animals they should proliferate in the host and be more easily detectable during the following screening.

Preparation of the aposymbiotic anemones

Step 2.

Some days before starting the actual infections screen all animals under a stereomicroscope with fluorescence capabilities and a filter set suitable to observe the algal chlorophyll fluorescence.

Use higher magnifications (around 20x total) to ensure not to miss any alga in the animals (fluorescence signals get brighter the higher the magnification is).

Preparation of the algal cells

Step 3.

Determine the cell density in the *Symbiodinium* culture you will use for infection.
Manual counting in a hemocytometer or automated counting by a flow cytometer work both fine. ***Count only intact and pigmented cells!***

Cell density of the culture should be somewhere between 100,000 and 1,000,000 cells/ml.
Here is a nice tutorial for counting with the hemocytometer: https://bitesizebio.com/13687/cell-counting-with-a-hemocytometer-easy-as-1-2-3/

Preparation of the algal cells

Step 4.

The final concentration of *Symbiodinium* cells in the anemone tank should be at least 10,000/ml. If doing infection experiments use exactly 10,000 cells/ml.

With a moderately dense culture this should only take a few milliliters.

Preparation of the algal cells

Step 5.

Transfer the appropriate culture volume into a centrifuge tube and spin the cells down for 10 min at 5000 ×g, resuspend the pellet in 1 ml of artificial sea water.

Preparation of the algal cells

Step 6.

Centrifuge again at 5000 ×g for 10 min and resuspend the pellet in 1 ml of artificial sea water.

Colonization

Step 7.

Place the anemones with the algae over night to 27°C and 12 h light:12 h dark.

Longer incubation time has usually no impact on infection efficiency.

Colonization

Step 8.

The next day, feed freshly hatched brine shrimps to the anemones.

The feeding strongly enhances Symbiodinium uptake. It might increase the efficiency if feeding happens parallel to addition of algae at step 7. Instead of living brine shrimps homogate of frozen brine shrimps might be used and enhance the infection.

Colonization

Step 9.

After 2-6 h clean the tank and change the water.

Place the tank back into an incubator with 12 h:12 h light:dark cycles and 27°C.

Colonization

Step 10.

For colonization experiments take samples in intervals of several days over a time course of 30 days with regular water changes but no feeding.

If the colonization is not part of an experiment, feed the anemones one to two times per week with brine shrimps and change the water afterwards.

Depending on the *Symbiodinium* strain and general condition of the anemones, proliferation of algae inside the host animals should be visible between two and 10 days after infection under a fluorescence stereomicroscope.

It will take more time before a change in Aiptasia coloration is visible by bare eye.
The reason for not feeding the animals during an experiment is that it could interfere with total protein measurements.

Warnings

Don't look directly into the light of a fluorescence light source!