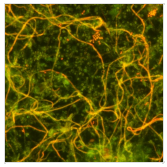

Version 1

Dec 01, 2020

Mollusk pedal mucus effects on epilithic biofilms V.1

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1 *Works for me* This protocol is published without a DOI.

The microscopic world, trophic and non trophic pathways in the intertidal rocky shore.

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MATERIALS TEXT

Laminar flow cabinet

Polycarbonate cell culture plate of 6-Wells

Aquariums with circulating seawater and constant aeration.

Sterile glass slide (20 x 7 cm)

1 L Sterile seawater

Sterile scalpel

Cryovials

0.01% acridine orange

500 mL PBS

Fluorescent Microscope with an excitation filter (FS38) 470/40 nm and emission filter 525/50 nm.

Image J

1 We cultured epilithic biofilms on cover glass slides inside a Polycarbonate cell culture plate of 6-Wells, for one week in K medium (Keller et al. 1987).

2 We collected, during nocturnal low tides, animals of each mollusk species from wave-exposed platforms and brought them to the laboratory in coolers and then placed to acclimatize for a week in separate aquariums by circulating seawater and constant aeration.

3 Collection of pedal mucus was done under a laminar flow cabinet (Connor 1986). Animals were carefully removed from their containers, washed in sterile-filtered seawater (0.2 µm pore-size filters), and then, each of them, was placed on an individual inclined sterile glass slide (21 x 7 cm).

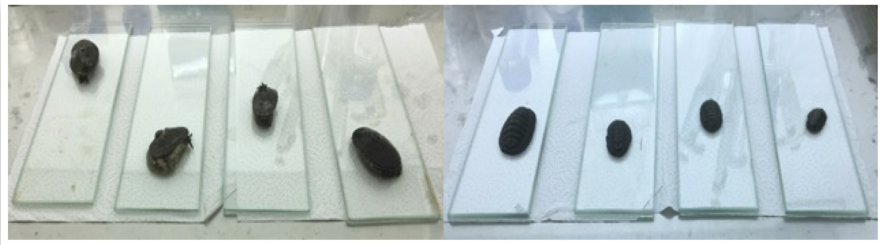

Fig 1. Sterile glass slide used for collection of pedal mucus.

4 Sterile seawater was added to stimulate individual movement and mucus production as they moved through the glass. The animals moved in the glass slides for a maximum of five minutes and then were removed.

5 The mucus on the replicate glass slides was removed with a sterile scalpel and put it in individual cryovials with sterile seawater.

6 The experiment consisted of placing the pedal mucus collected from the individuals, of each of the species, in separate replicated wells with cover glass slides that had been cultured with biofilm. The control wells received no mucus and treatments were randomly assigned to the cell culture plates.

7 After one week, we stained the cover glass slides with 200 µl 0.01% acridine orange for 5 minutes, and then we removed the biofilm. The acridine orange, AO, is a fluorescent compound that emit red fluorescence when attached to single-stranded templates (RNA) and green fluorescence if the nucleic acids are double-stranded (DNA) (Rigler 1966) and can be used to get gross estimates of the total amount of bacterial cells in the biofilm.
After 3 min incubation in the dark, the staining solution was removed, and the plate was washed twice with 500 µl of PBS solution.

8 We took five photographs in different fields of each cover glass slide under a Fluorescent Carl Zeiss AXIO Scope A1

Microscope using excitation filter (FS38) 470/40 nm and emission filter 525/50 nm.

9 Then, from the photographs, we measured the cover of the photosynthetic epilithic biofilm using the software Image J and the workflow:

a. Upload the image

b. Select: Image > Adjust > Color Threshold.

Parameters: Thresholding method = Default;
Threshold color = Red;
Color space = HSB;
Dark background (active).

c. Move the Brightness values until you select only the biofilm and not the background.

d. Select: Analyze >Measure, and copy the results in a table.

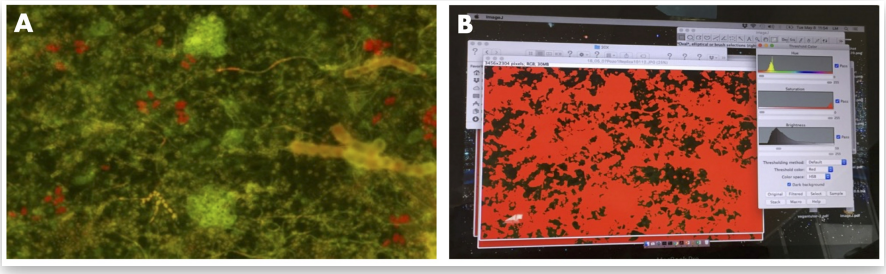

Fig 2. Cover measure of photosynthetic epilithic biofilm. A. Photograph of biofilms stained with 0.01% acridine orange. B. The same photograph analyzed with the workflow of Image J.

10 Covers under different treatments were analyzed with one-way ANOVA with grazer species as a fixed factor. A Tukey post-hoc test was performed to determine the pattern of differences.

LITERATURE CITED

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