Assessing Antifouling Activity of *Heteractis Magnifica* against *Amphibalanus Amphitrite* and *Escherichia Coli*

Abstract

A major problem for marine industries is biofouling, the accumulation of undesirable organisms on immersed artificial surfaces. Antifouling is used to inhibit biofouling, but many antifouling coatings with metallic properties cause harm to non-target species. In order to prevent harm to non-target species, natural bioactive metabolites can be used in antifouling coatings as opposed to metallic coatings. This research project focused on the sea anemone *Heteractis magnifica* due to past research that indicated the presence of natural antifouling agents in the respective organism. A barnacle settlement inhibition assay and biofilm assay were performed to determine the ability of the sea anemone crude extract to inhibit biofouling.

Introduction

The importance of using natural products for antifouling has been recognized because of their ability to inhibit biofouling organisms while remaining non-toxic to non-target organisms and the environment. Antifouling is necessary to prevent the growth of undesirable organisms. However, current antifouling coatings that consist of copper and other biocides cause lethal and sublethal effects on ecologically and economically important non-target species [3].

A potential source of natural antifouling agents is the sea anemone. Sea anemones contain secondary bioactive metabolites and nematocysts that show antimicrobial activity [1]. The respective toxins may contain antifouling agents since they are natural chemical repellents. Furthermore, toxins in sea anemones have been hypothesized to produce the bioactive compounds necessary to prevent parasitic or microbial colonization of their surface [2]. Due to easy availability of *Heteractis magnifica* and previous research that indicates that it is rich in potential antifouling agents, this organism was chosen to be screened.

Fig. 1 *Heteractis magnifica*

Purpose

The purpose of this study is to analyze the antifouling activity of *H. magnifica* through a larval barnacle inhibition assay and biofilm assay.

Hypothesis

Overall, the acetone extraction will show the most antifouling activity based on previous research of *Heteractis magnifica* crude extract.
Null Hypothesis: *Heteractis magnifica* crude extract will show no activity against the larval barnacle settlement and biofilm.

Variables

Control: Aged sea water treatment, LB
Negative Control: DMSO treatment
Positive Control: $CuSO_4$ treatment
Experimental Group: Crude extract treatments (25 µg/mL)
Independent Variable: Concentration of crude extract
Dependent Variables: Settlement and absorbance

Materials

- *Amphibalanus amphitrite*
- *Escherichia coli*
- *Heteractis magnifica*
- Multiskan FC Microplate Reader

Fig. 2 Materials for biofilm assay

Methods

Preparation of crude extract
- Entire body of *H. magnifica* homogenized
- Homogenate immersed in a beaker of methanol
- Process applied with separate pieces for ethanol and acetone extraction respectively; they were then centrifuged and evaporated to dryness

Larval barnacle culture
- *Amphibalanus amphitrite* nauplii were obtained from the field and reared in mass culture to cyprids
- Cyprids were collected from the culture by a sieve cascade, cleaned of debris and held at 4 - 6°C [regards to Daniel Rittschof and Beatriz Orihuela]

Larval Barnacle Settlement Inhibition Assay
- Before testing crude extract, a pre-test of larvae tolerance was performed
- Ten competent larvae were added by micropipette to each of the 6 replicates of 2 mL of the test solution
- $CuSO_4$ solution (25 µg/ml) was used as positive control and wells containing only filtered seawater with DMSO served as negative control
- The plates were incubated for a 24 h period under similar conditions as when the larvae reared
- Above procedure was repeated with methanol, ethanol, and acetone extracts

Antifouling activity against E. coli biofilm
- 96-well multi-well plate was filled with varying volumes of crude extract consisting of 10 µL, 20 µL, 30 µL, 50 µL, 100 µL, and 200 µL
- Another micro-well plate was prepared with fresh LB and 1 µL of the growth culture from the first plate was transferred to the second plate
- The supernatants from the wells were removed and the wells were washed using PBS and stained with 0.4% crystal violet
- A microplate reader was then used to analyze the plate spectrophotometrically

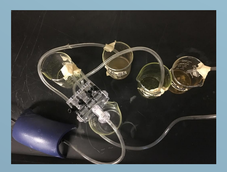

Fig. 3 Crude extract

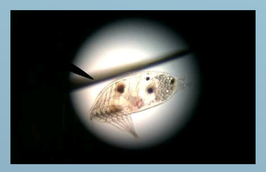

Fig. 4 Cyprid under microscope

Results

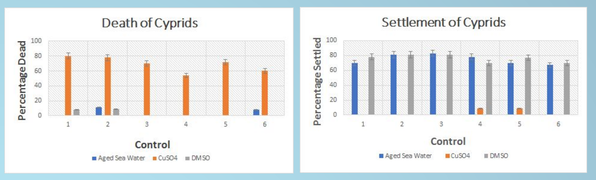

Fig. 5 The percentage of settlement and death is calculated for the controls of aged sea water, the positive control of $CuSO_4$, and the negative control of DMSO. There is no statistical significant change between the control and negative control, allowing for the crude extract to be dissolved in DMSO.

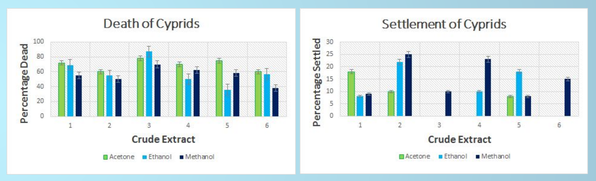

Fig. 6 The percentage of settlement and death is calculated for the acetone crude extract, ethanol crude extract, and methanol crude extract. The acetone is the most effective in inhibiting the settlement of cyprids and also has the highest average death rate.

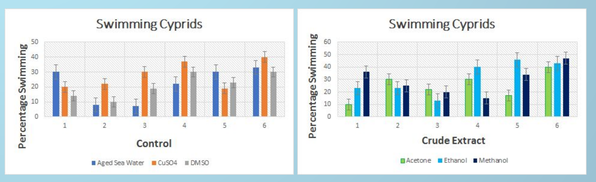

Fig. 7 The percentage of swimmers is calculated for the controls and crude extracts.

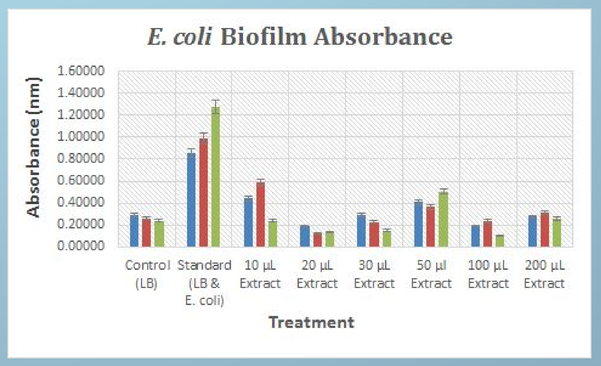

Fig. 8 Growth of *E. coli* biofilm in presence of LB, *E.coli*, and crude extract. Biofilm formation is impaired in the presence of the crude extract.

Discussion

The outcome obtained has the potential to provide an alternate antifouling agent to coatings containing copper. The inhibitory activity of the *H. magnifica* extract against larval barnacle culture represents an opportunity to discover anti-macrofouling agents from the extract. The acetone crude extract's death rate is more prominent than the methanol and ethanol crude extracts. The death rate of the cyprids for the acetone crude extract is similar to that of $CuSO_4$, indicating that there are powerful antifouling agents present in the acetone crude extract.

Furthermore, the acetone crude extract inhibited the growth of *E. coli* with 20 µl impairing the most formation of biofilm. The results indicate that *H. magnifica* crude extract is active against the two main groups of biofouling which are microfouling and macrofouling.

Conclusion

An active antifouling agent is present in the *H. magnifica* crude extract. It can be inferred that the active antifouling agent inhibits both macrofouling and microfouling because of its inhibitory effects on barnacle larvae and biofilm.

Further Work

Further experimentation would involve identifying the active agent through HPLC. Assays conducted in this experiment would be reproduced with the pure active agent identified.

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