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EFFECT OF CRUDE EXTRACT FROM MARINE POLYCHEATE CAPITELLA CAPITATA ON LARVAL SETTLEMENT OF BALANUS AMPHITRITE (CIRRIPEDIA, CRUSTACEA)

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ABSTRACT

Marine biofouling is a serious problem caused by the accumulation and settlement of microbial slimes, diatoms, barnacles, tunicates, bryozoans etc., on the hulls of seafaring vessels. In the present study, an attempt has been made to investigate the antifouling potential of marine Polycheates interaction against the settlement of cyprid larvae of *B. amphitrite*. The crude extract of Polycheate *Capitella capitata* significantly inhibited the cyprid larval settlement ($P<0.5$) than the control. The EC_{50} value of the crude extract (8 mg/ml) was lower than all the fractions. At higher concentration (16mg/ml), the crude extract of Polycheate *Capitella capitata* strongly inhibited the larval settlement.

KEY WORDS: **Biofouling, Polycheate *Capitella capitata, B. amphitrite***

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INTRODUCTION

Biofouling poses one of the most serious problems to marine industry and aquaculture development. In the marine environment, natural and artificial surfaces immersed in seawater are colonized by biofoulers including micro-foulers such as marine bacteria, algae, and protozoa, and macro-foulers such as barnacles, bryozoans, and tubeworms.[1] (Biofoulers that accumulate on the ship hulls increase drag and surface corrosion, thereby severely diminishing ships' maneuverability and carrying capacity.[2] In addition, biofouling causes huge material and economic costs in maintenance of mariculture, naval vessels, and seawater pipelines.[3] In order to control biofouling, broad-spectrum metal biocides, such as tributyl tin (TBT) and copper, have been added to marine paints as antifouling compounds. Although very effective, these biocides are often extremely toxic to a wide range of non-target organisms.[4] Extension of this research area is essential to identify novel effective nontoxic compound having potent anti-micro and macro fouling properties. These biogenic compounds could also be used effectively for future development of antifouling paints.[5] Naturally, marine environments harbor highly diverse microbial communities, which possess functionally undesirable and unexplored potentials, and they produce a variety of chemical deterrents for their defense purposes.[6] Marine natural products are a promising source of novel antifouling agents. Indeed, in the past few decades, many compounds with strong antifouling activity have been isolated from marine sponges, corals, and algae.[7,8.] In the present study, an attempt has been made to investigate the antifouling potential of marine Polycheates interaction against the settlement of cyprid larvae of *B. amphitrite*.

MATERIALS AND METHODS

Collection and extraction of marine Polycheate Capitella capitata

The Polycheate *Capitella capitata* were collected during the lowtide of the intertidal area of the west coast of Ratnagiri, India. The collected samples were rinsed with sterile sea water to remove associated debris and salt. Methanol and Methylene chloride extract of the Polycheate *Capitella capitata* was prepared as described by Rittschof *et al.*[9] The organic extract was fractionated by the Thin Layer Chromatography on silica gel. The extracts were fractionated using. The solvent system used was chloroform**:** methanol (9:1). Zone of separation were observed under ultraviolet florescence using 230-240nm and 250-270 nm lamp. Separated material was recovered from the plates by scraping and eluted with HPLC grade methanol. Methanol was removed by rotary evaporation under vacuum for using them for the antibarnacle activity.

Collection and rearing of barnacle cyprid larvae

Barnacles *B.amphitrite* was collected from west coast of Ratnagiri, India. Adult barnacles released the first stage nauplii and the positively phototrophic nauplii were collected in the filtered and sterilized sea water containing antibiotics. The young nauplii were fed daily with microalgae *Dunaliella tertiolecta*. The rearing vessels were kept in 28º C and 15:9h (L: D) photoperiod.

Settlement Assay

Barnacle settlement assays were undertaken using the method by Rittschof *et al*[9]. Briefly, plastic dishes were filled with 5.0 ml of filtered sea water at salinity of 33-35 ppt into which 3-day old cyprid were added. Settling test was performed using sterile Petri dishes and different concentrations of Polycheate *Capitella capitata* were coated (from 8 mg/ml, 10 mg/ml, 12 mg/ml, 14 mg/ml and 16 mg/ml). After drying, the experimental Petri dishes were filled with (5 ml) filtered seawater for 3 days and the dishes were replenished with 5 ml of fresh filtered seawater and then 20 cyprids transferred to each and assayed for settlement after 3 days. After incubation at 28º C for 9 h, the dishes were examined under the dissecting microscope to determine if there was any

mortality. The larvae were then killed with a few drops of 10% formalin and attached and unattached larvae were counted. Settlement data were expressed as the percentage of the larvae attached to the bottom of the dish. Effective concentration (EC_{50}) values were then calculated.

Statistical Analysis

All the experiments were performed in triplicates to ensure probability and reproducibility of the results. One-way ANOVA analysis was used to test for significant differences between the concentrations of Polycheate *Capitella capitata* on antifouling activity against fouling barnacle bioassay.

RESULTS

Cyprid Settlement Inhibition Assay

The crude extract of Polycheate *Capitella capitata* significantly inhibited the cyprid larval settlement ($P<0.5$) than the control. The EC_{50} value of the crude extract (10 mg/ml) was lower than all the fractions. At higher concentration (14mg/ml), the crude extract of Polycheate *Capitella capitata* strongly inhibited the larval settlement.

DISCUSSION AND CONCLUSION

Biofouling causes serious problems for marine industries and navies around the world[3]. Marine biofouling is a complex assemblage of organisms on artificial structures comprising micro- as well as macro- foulers and often it has been reported that micro-fouling facilitates macro-fouling process[10].Some marine organisms such as corals, algae, sponges, and ascidians have been shown to produce antifouling substances which in nature maintain them free from undesirable encrusting organisms[1,8,11]. The biochemical mechanisms that Polycheates have developed as a chemical defense for the growth inhibition of epiphytic micro and macro organisms might comprise a potential alternative for the prevention of biofouling. In this regard, sessile, soft bodied marine organisms maintaining a clean surface were identified as possible sources of natural product antifoulants (NPAs). Polycheates, with their rich chemical defense mechanisms are one of the most studied organisms for the isolation of NPAs. Natural products and their synthetic analogs exhibiting anaesthetic, repellent and settlement inhibition properties, but non-toxic to the non-target organisms, are preferred as potential antifouling agents[12]. Possible antifouling properties of the compounds isolated from the sponge was first recognized by Bakus[13]. Further studies in this direction have revealed the tremendous antifouling potential of some of the bioactive metabolites inherent in the sponges[2]. The antifouling strategy of Polycheate *Capitella capitata* was tested in the laboratory on larval settlement. The crude extract of Polycheate, *Capitella capitata* significantly inhibited the cyprid larval settlement ($P<0.5$) than the control. The EC_{50} value of the crude extract (10 mg/ml) was lower than all the fractions. At higher concentration (14mg/ml), the crude extract of Polycheate, *Capitella capitata* strongly inhibited the larval settlement. The settlement inhibition assays using barnacle cyprids have been used routinely to examine the antifouling properties of synthetic and natural compounds. Effect of crude extract of Polycheate, *Capitella capitata* barnacle setting was performed with *Balanus amphitrite* larvae and the results exhibited significant inhibition of the settlement of *B. amphitrite* cyprids. When the cyprids were exposed to various concentrations of crude extract of Polycheate, *Capitella capitata* ranging from 10, 12, and 14 mg/ml larval settlements was inhibited in a dose-dependent manner. EC50 was between 10 to 100%. This compound proved to have outstanding antifouling activity even at low concentrations. It meets many criteria to be a good candidate for a low-toxic/non-toxic antifouling additive and has been filed for its application for antifouling purposes.

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