



## Polycheate *Lumbrineris hetropoda* as antifouling compounds

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### Abstract

The antifouling activity of bioactive compounds from marine Polycheate *Lumbrineris hetropoda* studied. Larvae of *Balanus amphitrite* (Cyprids) were used to monitor the settlement inhibition and the extent to which inhibition was due to toxicity. The crude extract of Polycheate *Lumbrineris hetropoda* showed significant inhibition over the settlement. *Lumbrineris hetropoda* extract has slightly inhibited larval settlement with EC<sub>50</sub> value 12mg/ml. The high but variable levels of antifouling activity in combination with less amount of toxicity showed the potential of these metabolites in environmentally-friendly antifouling preparations. The settlement inhibitors from Polycheate *Lumbrineris hetropoda* could provide useful insight in to the mechanisms to control the larval settlement.

## INTRODUCTION

The marine industry and aquaculture development is facing serious problems that are posted by biofouling. 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. Dobretsov *et al.*, (2011) noticed that biofoulers accumulating on the ship hulls increase drag and surface corrosion, thereby severely diminishing ships' maneuverability and carrying capacity. Chambers *et al.*, (2006) stated apart from this biofouling causes huge material and economic costs in maintenance of mariculture, naval vessels and seawater pipelines. Yebra and Johansen (2004) observed Tributyl tin (TBT) and copper have been added to marine paints as antifouling compounds in order to control biofouling and broad-spectrum metal biocides. Although very effective, these biocides are often extremely toxic to a wide range of non-target organisms. Konstantinou and Albanis

(2004) identify novel effective nontoxic compound having potent antimicro and macro fouling properties. These biogenic compounds could also be used effectively for future development of antifouling paints. Naturally, marine environments harbor highly diverse microbial communities possess functionally undesirable and unexplored potentials producing a variety of chemical deterrents for defense purposes. Paul and Puglisi (2004) recognized marine natural products are promising source of novel antifouling agents. Qian P Y *et al.*, (2007) observed many compounds with strong antifouling activity have been isolated from marine sponges, corals, and algae in the past few decades.

To understand larval barnacle settlement, is the main objective of this investigation with the practical goal of developing non-toxic antifouling agents by the extract from Polycheate *Lumbrineris hetropoda*. An attempt has been made to investigate the antifouling potential of marine Polycheates interaction against the settlement of cyprid larvae of *B. Amphitrite* is focused in the present study.

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 Harder et al. 2003. The biochemical mechanisms that sponges 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). Sponges, with their rich chemical defense mechanisms are one of the most studied organisms for the isolation of NPAs (Thakur and Anil 2000). Sponges and Octocorals contain a wealth of secondary metabolites (Tilvi et al., 2004). 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. Possible antifouling properties of the compounds isolated from the sponge was first recognized by Bakus et al. (1983). Further studies in this direction have revealed tremendous antifouling potential of some of the bioactive metabolites inherent in the sponges (Chambers et al. 2006). Notable among them is polymeric alkylpyridinium salts (Poly-APS), a non-toxic NPA from the sponge *Reniera sarai* (Turk et al., 2007).

In the present study, an attempt has been made to investigate the antifouling potential of marine polychaetes *Lumbrineris hetropoda* interaction against the settlement of cyprid larvae of *B. amphitrite*

## MATERIALS AND METHODS

### *Collection and extraction of marine Polychaete Lumbrineris hetropoda*

The Polychaete *Lumbrineris hetropoda* were collected during the low-tide 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 Polychaete *Lumbrineris hetropoda* was prepared as described by Rittschof et al. (1985). The organic extract was fractionated by the Thin Layer Chromatography on silica gel. The extracts were fractionated using. The solvent system used was chloroform: methanols as the zone of separation were observed under ultraviolet fluorescence using 230-240nm and 250-270 nm lamp. The 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 28° C and 15:9h (L: D) photoperiod.

### **Settlement Assay:**

Barnacle settlement assays were undertaken using the method by Rittschof et al., (1985). Approximately 50-100 cyprids were placed to polystyrene container containing 5ml sea water as control or 5 ml desired concentration of vacuum dried test material. From two to six concentration of test solution, each with replicates was rested to compare the frequency of attachment in experimental solutions with attachment in the controls. Test petridishes were incubated for 22hr at 28°c and 15:9 Light: dark regime. Attached and unattached cyprids were counted

### **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 Polychaete *Lumbrineris hetropoda* on antifouling activity against fouling barnacle bioassay.

**RESULTS AND DISCUSSION**

**Table 1: Percentage of Settlement inhibition and Lethality of polychaetes *Lumbrineris hetropoda* at different concentrations**

Conc. of extract (Mg/ML)	No. of Cyprids used	% of mortalityLD <sub>50</sub>	Nos. of Cyprids settled(EC <sub>50</sub> )
8 mg/ml	100	70	70
10mg/ml	100	60	65
12mg/ml	100	50	48
14mg/ml	100	30	33
16mg/ml	100	100	20

The crude extract of Polycheate *Lumbrineris hetropoda* significantly inhibited the cyprid larval settlement (P<0.5) than the control. It showed antifouling activity with EC<sub>50</sub> value ranging from 16mg/ml and they were also lethal to cyprids with LD<sub>50</sub> value 12mg/ml. *Lumbrineris hetropoda* extract has slightly inhibited larval settlement with EC<sub>50</sub> value 12mg/ml. The extract showed promising activity, they were also toxic to 14mg/ml.

Biofouling is causing serious problems for marine industries and navies around the world Yebra and Johansen (2004). Marine biofouling is a complex accumulation of organisms on artificial structures comprising micro along with macro foulers. Callow and Callow (2002) has reported that micro-fouling facilitates macro-fouling process. Harder *et al.* (2001) proved the marine organisms such as corals, algae, sponges and ascidians are useful to produce antifouling substances which maintain from undesirable encrusting organisms. The biochemical mechanisms that Polycheates have developed as a chemical defense for the growth inhibition of epiphytic micro and macro organisms comprise a potential alternative for the prevention of biofouling. In this regard, sessile, soft bodied marine organisms maintain clean surface are identified as possible sources of Natural Product Antifoulants (NPAs). Polycheates, as rich in chemical defense mechanisms are one of the most studied organisms for the isolation of NPAs. Omae (2003) studied the Natural products and their synthetic analogs exhibiting anesthetic, repellent and settlement inhibition properties but non-toxic to non-target organisms are preferred as potential antifouling agents. The Possible antifouling properties of the compounds were isolated from the sponge was first noticed by Bakus *et al.* (1983). The further studies in this direction revealed the tremendous antifouling potential of some of the bioactive metabolites inherent in the sponges Chambers *et al.* (2006).

The antifouling strategy of Polycheate *Lumbrineris hetropoda* was tested in the laboratory on larval settlement. The crude extract of Polycheate *Lumbrineris hetropoda* has significantly inhibited the cyprid larval settlement (P<0.5) than the control. It has shown antifouling activity with EC<sub>50</sub> value ranging from 16mg/ml and they were also lethal to cyprids with LD<sub>50</sub> value 12mg/ml. *Lumbrineris hetropoda* extract slightly inhibited larval settlement with EC<sub>50</sub> value 12mg/ml. The extract also showed promising activity and were also toxic to 14mg/ml. This compound has proved the outstanding antifouling activity even at low concentrations. It meets many criteria for a low-toxic/non-toxic antifouling additive and its application for antifouling purposes. A study carried out by Holmstrom *et al.* (1996) showed the capacity for the marine bacterial strain *Pseudoalteromonas tunicate* for inhibiting the growth of common biofouling diatom *Amphora sp.* The field study of Olivier *et al.* (2000) concluded that the cyprids preferred unfilmed over biofilmed surfaces, which was in accordance with the present analysis. It has been widely reported that many bioactive natural products from marine invertebrate have striking similarities to the metabolites of their associated microorganisms, including bacteria (Proksch *et al.*, 2002). Thus, the present study highlighted the possible role of Polycheate *Lumbrineris hetropoda* associated providing an alternative to commercial metal-based antifouling coatings that have been believed to be environmental hazards due to their toxicity.

The settlement inhibitors from Polycheate *Lumbrineris hetropoda* could provide useful insight in to the mechanisms to control the larval settlement. Polycheate *Lumbrineris hetropoda* is a nontoxic settlement inhibitors could be of potential value as alternatives to the ecologically damaging toxic chemicals incorporated into paints to prevent the fouling.

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