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Short research note

Antifouling activity of bioactive substances extracted from *Holothuria scabra*

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Abstract

Methanol extract of *Holothuria scabra* was tested for antifouling activity using 'mollusc foot adherence bioassay'. It was found that the secondary metabolites of *H. scabra* effectively prevented foot adherence of *P. vulgata* at various concentrations. Based on the present findings it could be inferred that the bioassay guided purification and fractionation may give forth potent novel antifouling compounds.

Fouling organisms cause serious problems in the cooling water systems of power stations and culture structures for oysters, seaweed, fish, etc., and a lot of effort has been made to find efficient antifouling substances. Fouling organisms usually do not colonize on the surfaces of sponges, echinoderm and corals since these organisms produce potent secondary metabolites including specific deterrents (Selvin, 2002). The mechanisms of secondary metabolites synthesis and chemical ecology are highly complicated and less explored. Marine animals can accumulate toxic substances released from either dead or living red tide organisms directly from the water or by eating other toxic diets through the food chain (Bakus, 1968; Bakus and Green, 1974; Bakus, 1982). The *Holothuria* contain rich sources of the toxic metabolite, holothurin or saponin (Jayasree et al., 1991). These toxic deterrents might form a rich source for developing potent novel antifoulants. This paper deals with the details of the findings of antifouling properties of secondary metabolites isolated from *H. scabra.*

Specimens of *H. scabra* were collected from seaweed infested rocky substratum off Muttom coast (southeast coast of India). The collected live specimens were transported to the laboratory in a 250 l high density plastic tank provided with constant aeration. They were killed under ice and washed in fresh water and dissected to remove the internal contents. For the isolation of secondary metabolites, the tissue was minced in a homogenizer (Omni, U.S.A.) and extracted three times with methanol and methanol-dichloromethane (1:1). The combined extract was filtered and concentrated in a rotary vacuum evaporator (Buchi) at 40 °C.

The antifouling property of the isolated secondary metabolite was evaluated using 'foot adherence bioassay' developed by Selvin & Lipton (2002). The assay plates (100 mm petri plates) were spread evenly with 1 ml of extract, which was subsequently evaporated to dry in a hot air oven at 40 °C. The plates were filled one-third with seawater without any disturbance to the extract layer. The limpets (*Patella vulgata*) were removed carefully from the tank and introduced into the triplicate experimental plates at the rate of 5 animals per plate. The immediate foot reflex and mobility were monitored continuously for until the foot shrunk. Based on the adherence (fouling) or shrinkage of the foot, the fouling rate was estimated. After the com-

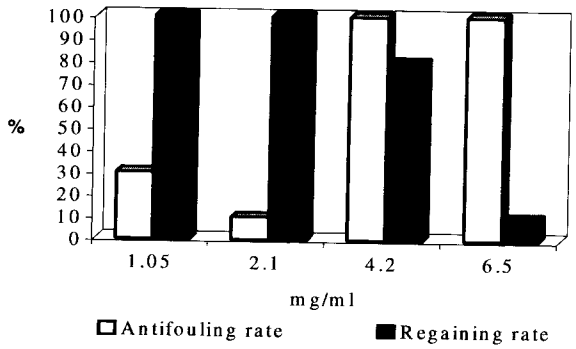

Figure 1. Antifouling activity of *Holothuria scabra*

pletion of exposure period, the treated animals were introduced in fresh seawater to observe their regaining rate.

In the present investigation, the secondary metabolite obtained from *H. scabra* was more potent in preventing the foot adherence of *P. vulgata*. The complete inhibition of foot adherence/fouling was observed at a concentration of 4.2 mg/ml (Fig. 1). This concentration was considered as a safe dose as 80% of the exposed *P. vulgata* were regained after the test period. However *H. scabra* was relatively more toxic at 6.5 mg/ml, in which all the experimental animals were died.

As the submergible methods are highly time-consuming and require substantial quantity of test compounds, reliable and effective primary assay systems are prerequisite for developing potent novel antifouling secondary metabolites. Although settlement-based microassay was used (Rittschof et al., 1985) for such purpose, the protocol based on the adult organisms was scanty. The reflex-based conventional 'foot stimulating' assay using the blue mussel *Mytilus edulis galloprovincialis* (Sera et al., 1999 a, b) was found ineffective. The foot was found stimulated even with seawater in many attempts made in our laboratory. Considering this lacuna, the assay was modified with a new 'foot adherence' technique to determine the activities of potent marine natural products. By principle, the fouling organisms require a suitable surface for their foot adherence. The attachment process involves recognition of the surface and production of biological adhesives that ensure attachment. Based on this principle, the present assay was developed, which was more effective over the conventional assay technique. Using the present bioassay system, potent antifouling substances were successfully isolated from sponges and seaweeds (Selvin, 2002).

Toxicity seems to help sessile or slow moving forms (Bakus et al., 1986). *H. scabra* also seems to be protected in this manner. *H. atra* metabolites were toxic to fish, mice and haemocytes (Rao et al., 1985). The pink glands referred to as 'Cuverian glands' of holothurians contain toxic substance, holothurin characterized as saponin (Nigrelli, 1952), which was found to be responsible for the potent toxicity or bioactivity. However according to Matsuno & Ishida (1969) saponins are found in all parts of *H. lecospilota*. Other researchers have also reported the presence potent antifouling secondary metabolites in various holothurian species. According to Mokashe et al. (1994), the methanol extract of a *H. leucospilota* effectively prevented the growth of biofilm forming marine diatoms, *Navicula subinflata* and *N. crucicula*. The antifouling property of the extract of *H. leucospilota* was found to be species specific (Gonsalves, 1997). According to Nagabhushanam et al. (1994), the toxic effect and the 96 h LC50 values of *H. leucospilota* towards *Caridina rajadhari* decreased with increasing exposure period. The similar trend of increasing toxicity with time was observed in the present experiment. Therefore the bioassay guided purification and fractionation may give-forth potent antifoulants. The excess toxicity can be minimized through alteration of the functional groups.

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