intersection sites of Delhi. Higher NO_2 values observed in the present study appear to be on account of rapid growth in the number of automobiles in Delhi during the intervening period between 1997 and 1999. NO_2 values at CPCB regular monitoring sites were close to the NO_2 values observed at sites in the present study, S1, S3, S5, and S7, which were away from the direct impact of any busy traffic corridor.

High levels of NO_2 observed in the present study suggest that that there is a need for comprehensive NO_2 monitoring network, including urban, peri-urban, rural and remote areas in order to determine the effect of NO_2 on crop plants, human beings and to also design pollution control strategies. Results of this study have demonstrated the convenience of passive samplers for the measurement of NO_2 in ambient environment in a highly cost-effective manner. Passive samplers are simple, cheap and efficient devices, which operate without any power source and do not need elaborate calibration and maintenance. They are found to be ideally suited for developing wide spatial monitoring network of NO_2 in a highly cost-effective manner covering urban, peri-urban, rural and remote areas.

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Development of a rapid 'mollusc foot adherence bioassay' for detecting potent antifouling bioactive compounds

J. Selvin and A. P. Lipton*

Central Marine Fisheries Research Institute, Thiruvananthapuram 695 521, India

Developing potent antifouling compounds from marine organisms has several R&D applications. The 'foot stimulating bioassay' for assessing the antifouling activity suffers interference with the reflex system of test organisms. The assay was modified with a new, rapid 'mollusc foot adherence technique' in order to measure the activities of potent marine natural products (MNPs). The common limpet, *Patella vulgata* was used as the test organism to detect the foot adherence. In the experiments with methanol extract of holothuria, *Holothuria scabra*, the foot adherence of *P. vulgata* was completely prevented at a concentration of 4.2 mg/ml. For detecting the potent activity, the assay plates require minimum quantity of test compound and the assay can be completed within one hour. In addition, after the evaluation of activity, the relative toxicity can also be estimated based on the per cent regaining ability of test organism in fresh seawater. This will lead to a rapid and sensitive evaluation of the MNPs when compared to the conventional 'foot stimulating bioassay' system.

IN view of serious fouling problems in the cooling systems of power stations, and culture systems of oysters, seaweed and fish, constant efforts are being made to screen efficient antifouling substances. Although formulations of copper and organotin have been used effectively against these foulers, there is a concern over the impact of such chemicals on the environment. Alternate less toxic but more potent compounds have to be screened that the fouling organisms do not colonize on the surfaces of sponges, echinoderms and corals, as they may produce potent secondary metabolites including specific deterrents[1–5].

In addition to screening potent products, it is also required to develop reliable and rapid *in vitro* assay systems for the bioassay-guided purification and fractionation processes. Of the known bioassay procedures, the common one is the conventional 'submerged method'. This method is advantageous, but requires a month or even more to obtain reliable results. In order to reduce the test period and to detect antifouling properties, a rapid 'mollusc foot adherence assay' was developed using the common limpet, *Patella vulgata*. The limpet

*For correspondence. (e-mail: liptova@yahoo.com)

adheres firmly to rocky surfaces or other hard substrata by the grey-green foot with a broad flat adhering surface. Considering its firm adhering nature and extensive colonization causing fouling and biodeterioration of submerged structures, it was chosen as a test organism to develop an effective *in vitro* model for evaluating potent anti-fouling compound(s) extracted from marine organisms. This paper describes the modified 'foot stimulating bioassay' for screening antifouling substances.

Specimens of *P. vulgata* were collected from the rocky surfaces off Kovalam coast, Thiruvananthapuram, Kerala (southwest coast of India). The healthy specimens were segregated and transported to the laboratory in a container provided with seaweed, *Ulva fasciata*. In the laboratory, they were maintained in 1000 l-capacity FRP tanks with thoroughly cleaned sand bed studded with *U. fasciata* and the tanks were well aerated. They were fed *ad libitum* with the algae *U. fasciata* in the tank. The hydrological conditions were monitored continuously. After 7 days of acclimatization, the limpets were used for the bioassay.

The assay procedure is depicted in Figure 1. The assay plates (100 mm petri plates) were spread evenly with 1 ml of methanol extract/fraction of *Holothuria scabra*, which was subsequently evaporated to dryness in a hot air oven at 40°C to obtain uniform film of the extract/fraction. These plates were filled up to one-third with filtered seawater without any disturbance to the extract layer. They were kept on illuminated glass surface to observe the foot reflex. The limpets were removed carefully from the tank and introduced into the experimental plate (triplicates) at the rate of 5 animals per plate. The immediate foot reflex and mobility were monitored continuously until the foot was completely shrunken. The limpets, which attempted to get attached onto the surface of others, were dislodged using a glass rod. As the attachment process involves recognition of a suitable place to spread their foot for adherence, the percentage of fouling was estimated based on the spreading or shrinking of the foot. After the completion of exposure period, the treated snails were removed and introduced into another petri dish in which fresh filtered seawater was kept to observe the percentage of recovery. The regaining capacity was determined by comparing the number of snails, which spread their foot compared to the total snails initially introduced. Based on the recovery percentage, the LD_{50} values were also calculated. The results of antifouling activity of secondary metabolites isolated from the holothurian *H. scabra*[6] based on this technique are presented in Table 1.

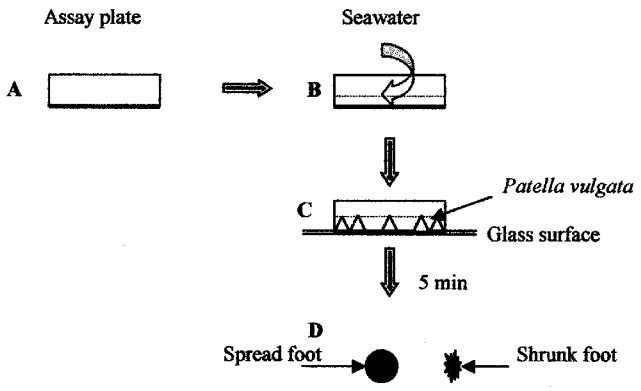

Figure 1. Procedure of the foot adherence assay: **A**, Preparation of the assay plate with 1 ml of selected concentration of extract/fraction; **B**, Sea water was filled without disturbance to the extract layer; **C**, The limpets were removed carefully from the tank and introduced into the experimental plates in triplicate and kept on an illuminated glass surface to observe the foot reflex; **D**, Based on the foot adherence or shrinkage, the fouling rate can be estimated.

The complete inhibition of foot adherence/fouling was observed at a concentration of 4.2 mg/ml (4% level) within 10 min. At the concentration of 6.5 mg/ml, it was relatively more toxic and led to the death of 90% of experimental snails. In general, toxicity seems to help the sessile or slow-moving forms[7]. The present study also confirms that *H. scabra* in its habitat is protected in this manner. The pink glands referred to as 'Cuverian glands' contain a toxic substance, holothurin, characterized as saponin[8,9]. Saponin was reported to be responsible for the potent toxicity or bioactivity. Therefore the toxicity and antifouling activity observed in the extracts could be due to the presence of such toxins. The excess toxicity can be minimized through alteration of functional groups. With the present simple assay system-guided purification and fractionation, it may be possible to evaluate potent antifoulants. With the present bioassay, the successful evaluations of antifouling substances from marine sponges such as *Dendrilla nigra*, *Axinella donnai* and *Clathria gorgonoides* were also reported[6].

As the submergible methods are highly time-consuming and require substantial quantity of test compounds, reliable and rapid primary assay systems are very important for developing potent novel antifouling secondary metabolites. Although settlement-based micro assay was used[1] 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*[10] was found ineffective in many attempts made in our laboratory. In this assay, when an active liquid stimulus, such as a solution of $CuSO_4$ or test compound was exposed on the foot, the mussel contracted its foot immediately. In the case of an inactive compound or control, the foot of the mussel showed no reaction or contraction even after a few seconds. However, during the preliminary experiments, it was found that the assay was suffering interference with the reflex system of test organisms because the foot contracted immediately even with the stimulus of seawater. Considering this lacuna, the technique was modified with this 'foot adherence' technique to determine the activities of potent marine natural products. By principle, the fouling

Table 1. Antifouling activity of secondary metabolites isolated from *H. scabra*

Concentration of extract (mg/ml)	Fouling (%)	Regaining (%)	Behavioural changes of snails observed during exposure and after in fresh sea water
1.05	72 ± 9.8	98 ± 6.0	Moving with spread foot
2.1	14 ± 4.9	94 ± 9.16	Attached with spread foot; regained immediately
4.2	0	78 ± 5.74	Shrunken foot; regained after 5 min
6.5	0	10 ± 4.26	Shrunken foot; mortality

Values in mean ± SD; $n = 10$.

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 have the following merits/advantages over the conventional assay technique:

- As the assayed snail is a common rock fouler, it could be easily collected from all over the coastal area.
- The test plates could be prepared with minimum quantity of test compound.
- The assay can be completed within an hour.
- Usually the snail finds a suitable place initially and spreads its foot for adherence. Therefore the rate of fouling could be easily monitored under a glass surface based on the reflex of foot adherence.
- After the experiment, the relative toxicity could also be estimated based on the regaining rate in fresh seawater.
- The mechanism of antifouling activity of the compounds on experimental *P. vulgata* may be due to the failure of recognition of suitable surface (due to sensory impairments) or inhibition of production of its biological adhesive required for attachment.

Considering the above merits over the conventional 'foot stimulating' assay, it could form a reliable alternate method for assessing the potent antifouling activity and bioassay-guided purification processes.

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Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria

Rambir Singh[†], Ramesh Chandra[†], Mridula Bose[#] and Pratibha Mehta Luthra[†,*]

[†]Dr B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110 007, India
[#]V.P. Chest Institute, University of Delhi, Delhi 110 007, India

***Curcuma longa* rhizome extracts were evaluated for antibacterial activity against pathogenic strains of Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) bacteria. Essential oil was found to be most active and its activity was compared to standard antibiotics gentamycin, ampicillin, doxycycline and erythromycin in these strains. Only the clinical isolate of *S. aureus* showed more sensitivity towards essential oil fraction than the standard strain. The use of essential oil from turmeric as a potential antiseptic in prevention and treatment of antibacterial infections has been suggested.**

CURCUMA longa, commonly known as 'turmeric', is widely used as a spice and colouring agent, and is known

*For correspondence. (e-mail: luthrapratibha@rediffmail.com)