Indian Journal of Marine Sciences
Vol. 23, March 1994, pp. 57-58

Growth inhibition of periphytic diatoms by methanol extracts of sponges and holothurians

S S Mokashe, A Garg, A C Anil & A B Wagh
Marine Corrosion & Materials Research Division, National Institute of Oceanography, Dona Paula, Goa-403 004, India
Received 30 August 1993, revised 20 January 1994

Crude methanol extracts of a holothurian *Holothuria leucospilota*, and two sponges *Craniella* sp. and *Ircinia ramosa* were tested for their inhibitory effects on the growth of two marine diatoms, *Navicula subinflata* and *Navicula crucicula*, by diatom plating method. Populations of both the diatom species expressed in terms of total chlorophyll-*a* values were found to be adversely affected by all the 3 test extracts. *Ircinia ramosa* extract amongst these exhibited maximum inhibitory effect on the growth of the diatoms.

A freshly-immersed surface in seawater undergoes series of changes resulting in the development of a microfouling community. In euphotic zone this community is dominated by microalgae, like autotrophic diatoms[1]. These diatoms rapidly colonise on oil platforms, ship hulls and buoys[2,3]. Their colonisation is generally followed by the settlement of invertebrate larvae[4]. These microalgal forms are reported to be highly tolerant to antifouling paints containing copper and tin[5,6]. The capability of these forms to grow over painted surfaces may afford protection to fouling larvae from toxic components of the surface coatings[7].

Studies on holothurians and sponges[8] have shown that they may either be toxic and/or posses effective defense behavioural pattern. This probably arises from bio-active compounds and may also possess antifouling properties. In pursuit of identifying environmentally safe antifouling material, methanol extracts of two sponge species (*Craniella* sp., *Ircinia ramosa*) as well as of one holothurian species (*Holothuria leucospilota*) were screened for their toxic efficacies. Two diatoms *Navicula subinflata* and *N. crucicula* were used in the bioassay. These soft-bodied organisms were collected from the intertidal rock pools at Ratnagiri, along central west coast of India (lat. 16°-62′ N and long. 73°-18′ S). Specimens of sponges and holothurians were washed thoroughly with fresh seawater before preserving them individually in methanol. The specimens were homogenised in a blender and this homogenised material was then extracted with 100% methanol by shaking for 12 h at room temperature. The extract was then centrifuged and the supernatant liquid was collected and filtered (Whatman-1 filter paper). The crude extract thus obtained was concentrated by using rotary evaporator under vacuum at 38°C and stored in a refrigerator.

The method of diatom plating was used for assessing the inhibitory effects of the extracts. Extract of each test organism (0.1 mg) was dissolved in 1 ml double distilled sterilized water mixed with 15 ml of agar medium[9]. It was then transferred to petri dish (9 cm diameter). Unialgal culture (1 ml) was evenly spread over the medium in each petri dish[10]. For each test six petri dishes were used. The changes in the diatom populations were determined in terms of total chlorophyll-*a* (chl-*a*) content ($\mu g.l^{-1}$) after 8 days exposure. Total chl-*a* content was estimated using spectrophotometer[11]. The petri dish content was extracted with 15 ml of 90 % acetone for 18 h. Mean value and standard error(SE) were calculated for all cases, so as to obtain values at 99% confidence limit[12]. The effect of extracts on diatoms is expressed as change in chl-*a* concentration relative to the control.

Chlorophyll-*a* values recorded for test plates containing *I. ramosa* extract (0.04 $\mu g.l^{-1}$ and 0.04 $\mu g.l^{-1}$) were lesser than the initial values recorded at the time of inoculation (0.75 and 1.14 $\mu g.l^{-1}$) (Fig. 1). The relative changes in chl-*a* concentrations with reference to control were 0.29 and 0.23% for *N. subinflata* and *N. crucicula* respectively (Fig. 2). Similarly, the values with extract of *Craniella* sp. were found to be 47 and 63%. In the case of *H. leucospilota* extract they were 11 and 19%. This indicates that influence of each extract on both the diatom species tested was nearly the same.

Examination of diatoms on test plates revealed that the cells of both the diatom species were partly degraded by *I. ramosa* extract. Diatoms in petri plates containing *H. leucospilota* extracts were aggregated

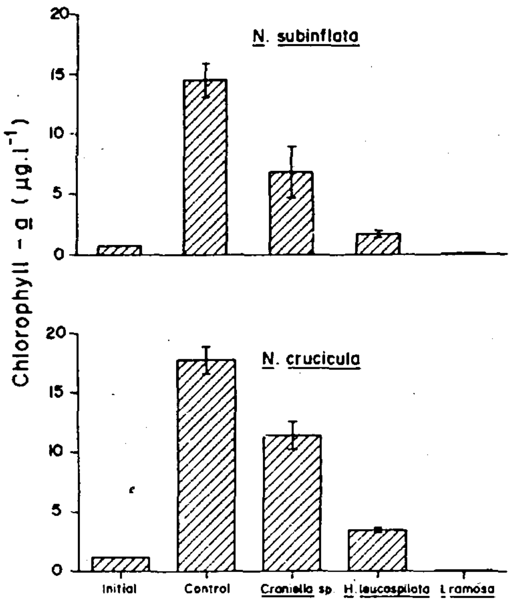

Fig. 1—Total chlorophyll-*a* content on plates of *Navicula subinflata* and *N. crucicula*

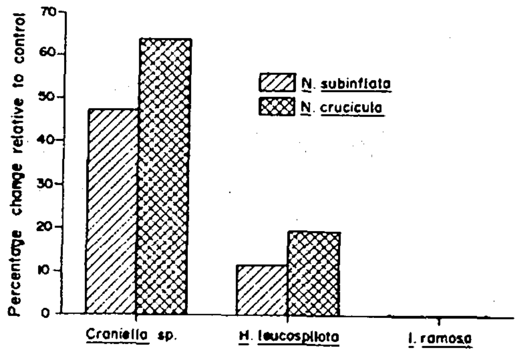

Fig. 2—Effect of crude extracts on chlorophyll-*a* content

in clumps. The diatoms in the presence of *Craniella* sp. test plates, showed reduced chl-*a* content, but the cells were morphologically normal. This suggests that the bio-active materials had varying effects on the diatom species used in the present assays. Earlier studies[13] also revealed that the crude methanol extract of *Craniella* sp. had a positive antibacterial activity against five bacterial strains at 50 μg. disc^{-1}.

The authors deeply appreciate the encouragement given by Director. This work is supported by the India—USA Sub-commission (PL-480, Grant no. N00014-90-J-1150).

References

1 Cooksey K E & Cooksey B, in *Algal biofouling*, edited by L V Evans & K D Hoagland, (Elsevier, Amsterdam) 1986, 55.
2 Evans L V, *Bot Mar*, 24 (1981) 167.
3 Characklis W G & Cooksey K E, *Adv Appl Microbiol*, 29 (1983) 93.
4 Marzalek D S, Gerchakov S M & Udey L R, *Appl Env Microbiol*, 38 (1981) 987.
5 Daniel G F & Chamberlain A H L, *Bot Mar*, 14 (1981) 123.
6 Callow M E & Evans L V, *Bot Mar*, 14 (1981) 201.
7 Fischer E C, Castelli V J, Rodgres S D & Bleile H R, in *Marine biodeterioration: An interdisciplinary study*, edited by J D Costlow & R C Tipper, (Naval Institute Press, Annapolis) 1984, 156.
8 Bakus G J, *Mar Biol*, 2 (1968) 23.
9 Targett N M, Bishop S S, McConnell O J & Yoder J A, *J Chem Ecol*, 9 (1980) 817.
10 Chan A T, Andersen R J, Le Blanc M J & Harrison P J, *Mar Biol*, 59 (1980) 7.
11 Strickland J D H & Parsons T R, *A manual of chemical and biological methods for seawater analysis*, 1984, 101.
12 Sokal R R & Rohlf F J, in *Biometry*, (W H Freeman, San Francisco) 1981, pp 859.
13 Mokashe S S, Tulaskar A V, Venkat K & Wagh A B, *Biofouling Control* (1994), (*in press*).