

### Short Communications

## Effect of holothurian and zoanthid extracts on growth of some bacterial and diatom species

Cynthia O L Gonsalves

Chemical Oceanography Division, National Institute of Oceanography, Dona Paula, Goa, 403 004, India

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The antifouling properties of the extracts from two zoanthids, viz. *Zoanthus* sp, *Protospalythoa* sp and one holothurian species, viz. *Holothuria leucospilota* occurring in the coastal waters off Goa were tested against 5 bacteria and 2 diatom species. All the three extracts possessed antifouling activity but their efficacy was found to be species specific. The highest bacterial inhibition zone ranged between 3-4 mm with both the zoanthid extracts. In case of diatoms, inducement of growth was observed in lower concentrations (200-400 ppm) and inhibition at higher concentrations (400 ppm and above). The results of the present study suggest that these marine natural compounds can possibly be used as antifouling agents.

Fouling is a widespread phenomenon causing serious maintenance problems of ship hulls, cooling systems and several other marine engineering facilities. To combat this menace, copper and organotin compounds are being used as chemicals in antifouling paints. These paints being deleterious to non-target life, necessitates the search for natural compounds of biological origin which are relatively free of chemical toxins<sup>1,2</sup> and are more species specific. It is known<sup>2</sup> that some marine invertebrates especially sessile forms are seldom covered by other organisms suggesting that they possess some bioactive compounds which prevent the settlement of the larvae of the later arriving species. In the search for newer approaches to antifouling, it is possible to mimic such organism's defensive faculties. The present work was therefore carried out to examine the antifouling properties of the compounds extracted from holothurians and zoanthids. Selected bacteria and diatoms were used as the test species in these bioassays.

Three species namely, *Holothuria leucospilota* Brandt (sea cucumber), *Zoanthus* sp and *Protospalythoa* sp (zoanthids) were collected from the rocky intertidal expanse of the Anjuna beach, Goa. The zoanthid colonies were removed from the rock surface using pincers and spatula while the holothurians were collected using big forceps. Specimens collected were rinsed well with fresh

seawater and brought to the laboratory. Methanol extracts were obtained from the whole body tissues and concentrated by vacuum evaporation. Crude extracts obtained were tested for their inhibitory effects on 5 species of marine bacterial isolates (*Bacillus cereus*, *B. circulans*, *B. pumilus*, *Pseudomonas vesicularis* and *P. putida*) and 2 species of diatoms (*Navicula subinflata* and *Nitzschia closterium*). The test bacterial cultures were obtained from the stock cultures maintained in the Microbiology Laboratory of National Institute of Oceanography, Goa. For confirmation of their adhesive nature, sterilised filtered seawater in 50 ml beakers were inoculated with the bacteria culture. Glass coverslips were kept floating on the seawater inoculated with the bacteria. After 6 hours the coverslips were removed and stained with gentian violet to check the adherence of bacteria. Only those cultures which formed a slime layer on the coverslips were chosen for the bioassay tests. The species identity of the chosen cultures were later confirmed using the Microbial Identification System (Hewlett Packard, USA).

The bacterial assays were carried out by the agar plate diffusion method<sup>3</sup>. Concentrations of 0.5 mg/6 mm disc of the extract prepared in methanol was introduced on sterilised filter paper discs (6mm). After solvent evaporation, the discs were laid on agar plates seeded with the test species. Controls were maintained in duplicate with solvent

alone. Four replicates of each concentrations were used in the assay and average values were recorded. Plates were incubated overnight at room temperature (~28°C). Growth inhibition zone was measured<sup>4</sup> in millimeters and scored as +++ = 3-4 mm, ++ = 2-3 mm, + = 1-2 mm. Inhibition zones measuring less than 1 mm were not scored. The bacterial assay results were compared with those obtained using standard antibiotic discs of penicillin-G (10 units/disc) and chloramphenicol (30 mcg/disc).

In the case of diatoms, seawater (~30 liters) collected from Dona Paula was filtered through 20µ nylon mesh. Phytoplankton samples thus collected were suspended in f/2 medium<sup>5</sup> in a 250 ml beaker. After 2 days, the medium holding the suspended cells was decanted and a fresh growth medium was added. The cells attached to the walls were then gently scraped with a fine brush and *Nitzschia closterium* and *Navicula subinflata* cells from these were picked up under microscope using fine capillary tubes. Subsequent subculturing and re-picking of cells was done until pure cultures were obtained. The method followed by Slattery *et al.*<sup>6</sup> was slightly modified for the bioassay, in that, instead of the tissue level concentrations of extracts, concentrations in terms of ppm are used in the present assay. Aliquots of each extract stock solution prepared in distilled water (1cc=50 mg) was taken corresponding to the desired concentrations to be used (200 - 1000 ppm). Actively growing cultures with an initial suspension (~1000 cells/ml) were used and the final volume adjusted to 50 ml using the growth medium in 125 ml Erlenmeyer flasks. All assays were carried out in quadruplets. Control flasks were without the extracts and contained only the cells in f/2 medium. Flasks were incubated at room temperature (~28°C, 12L:12D). Growth inhibition was monitored after 6 days in duplicate (i.e. during the exponential growth phase) in terms of the number of attached algal cells in a Sedgwick rafter<sup>7</sup> and expressed as cells/ml.

All the three extracts showed antibacterial properties in varying degrees and specificity (Table 1). The highest efficacy in terms of zone of inhibition was observed with *Zoanthus* sp and *Protospalythoa* sp extracts against *B. circulans* with inhibition zones of > 3 mm in diameter. Inhibition

in other bacterial isolates ranged from 1-2 mm. Bacterial inhibition with the standard antibiotic discs was found to be more effective than the extracts and produced greater inhibition zones (17 and 26 mm respectively of penicillin-G and chloramphenicol, Table 2).

In case of diatoms, the extracts induced growth at lower concentrations and caused inhibition at higher concentrations. Extracts of *H. leucospilota* and *Zoanthus* sp stimulated growth of *N. subinflata* at 200 ppm concentration, whereas with *Protospalythoa* sp extract, growth stimulation was observed at 400 ppm, which decreased at enhanced concentration of 600 ppm (Fig. 1). In *Nitzschia closterium*, growth stimulation was observed at 200 ppm with the extract of *Zoanthus* sp, whereas extracts of *H. leucospilota* and *Protospalythoa* sp showed only the inhibitory effects.

Saponins from holothurians are well known toxic components possessing ichthyotoxic, hemolytic, neurotoxic, cytotoxic, antitumor

Table 1—Results on inhibition of growth of the test bacteria with crude extracts of *H. leucospilota*, *Zoanthus* sp and *Protospalythoa* sp against film forming marine bacteria in terms of inhibition of growth (mm) at concentration of 0.5 mg/6mm disc.

Test organism	Whole body extracts		
	<i>H.leucospilota</i>	<i>Zoanthus</i> sp	<i>Protospalythoa</i> sp
<i>Bacillus cereus</i>	-	+	-
<i>Bacillus circulans</i>	+	+++	+++
<i>Bacillus pumilus</i>	+	+	+
<i>Pseudomonas vesicularis</i>	-	-	-
<i>Pseudomonas putida</i>	+	-	-

+ = 1-2 mm diameter; ++ = 2-3 mm diameter; +++ = 3-4 mm diameter, - = no inhibition

Table 2—Growth inhibition (mm) of bacteria using the standard antibiotic discs of penicillin-G (10 units/disc) and chloramphenicol (30 mcg/disc).

Test organism	Antibiotics	
	Penicillin-G	Chloramphenicol
<i>Bacillus cereus</i>	-	19
<i>Bacillus circulans</i>	7	26
<i>Bacillus pumilus</i>	-	14
<i>Pseudomonas vesicularis</i>	-	-
<i>Pseudomonas putida</i>	17	18

- = no inhibition

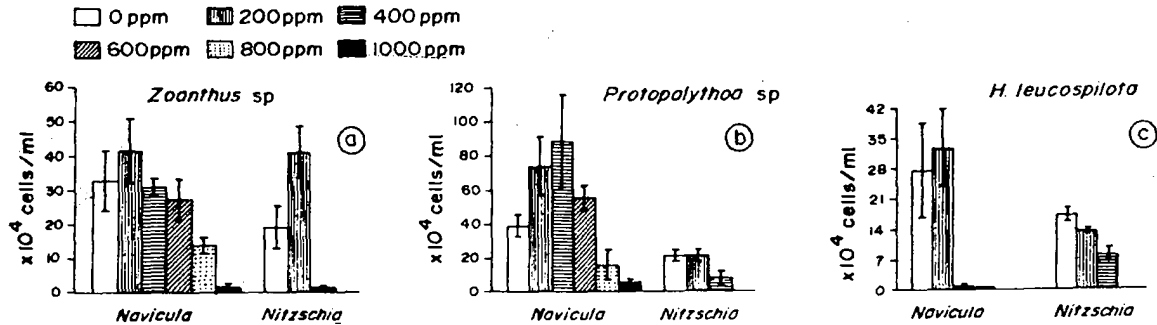


Fig. 1—Growth inhibition of the diatoms, *N. subinflata* and *N. closterium* in presence of the crude extracts of (a) *Zoanthus* sp (b) *Protopalpythoa* sp and (c) *H. leucospilota*.

activities<sup>8</sup>. Similarly, Palytoxin<sup>9</sup> isolated from the zoanths of *Palythoa* spp has cytotoxic properties and extremely active in cardiovascular systems. Paragracine from the zoanthid, *Parazoanthus gracilis* possesses antibradykinin, antihistamine and antiacetylcholine activities<sup>9</sup>. The present work adds to our knowledge on the antimicrofouling properties of crude extracts of *H. leucospilota*, *Zoanthus* sp and *Protopalpythoa* sp and could be deduced that the bioactive compounds responsible for these activities can possibly be used as antifouling agents.

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