

# Antimicrobial Properties of Poriferan Species from Indian Waters

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Poriferan species belonging to Class Demospongiae were collected from the rocky intertidal pools of Ratnagiri and extracted with methanol. The methanol extracts were tested for antimicrobial activity against six fouling bacterial strains. Extracts from *Craniella* sp. at concentrations of 50 µg/disc (7 mm diameter) were positive against all strains, suggesting the use of these extracts for further antifouling studies.

The use of marine bioactive substances as natural antifouling agents has the advantage of being nontoxic to the marine environment. It is known that organisms such as ascidians, sponges, soft corals, holothurians, and sea anemones are free of epibionts and fouling organisms, even though they are exposed to a variety of algal spores and invertebrate larvae.

Nagrelli et al. (1959) first reported antimicrobial activity in sponges. Since then many authors (Burkholder et al. 1973, Bakus and Green 1974, Green 1977, Bergquist and Bedford 1978, Bakus and Thun 1979, Thompson et al. 1985, Joseph and Ara 1991) recorded chemical compounds active against microorganisms from a variety of marine sponges. The possible antifouling properties of compounds isolated from sponges was first recognized by Bakus et al. (1983). A perusal of the literature revealed that only Sarma et al. (1991) evaluated the antifouling properties of Indian marine sponges. This paper provides insight on antifouling compounds from different soft bodied marine organisms. After preliminary examinations with several species, *Craniella* sp. belonging to the Choristida group was selected because it had antimicrobial activity.

## METHODS

The sponge material used in this investigation was collected from the rocky intertidal pools of the Ratnagiri coast (16°62', 73°18'). The material (2.5 kg) was thoroughly washed with fresh seawater, weighed, added to a liter of methanol, and then brought to the laboratory at Goa. The methanol was decanted after one day, the material homogenized in fresh methanol, and kept for two days. Following centrifugation at 2,500 rpm for one hour, the supernatant was added to the initially decanted methanol. This extract was concentrated using a rotary evaporator and brought to a final volume of 125 ml.

The bioassay organisms were isolated from microfouling films developed on aluminum and glass panels exposed for two days at a depth of one meter. Bacterial cultures were grown in nutrient marine agar broth overnight and 0.5 ml samples of the cultures were spread on agar test plates. An aliquot of each concentration (25 and 50 µg/disc) of extract was then applied to a sterile Whatman No. 1 filter paper disc (7 mm). Control discs were made by using methanol only. All discs were allowed to dry and were then placed on agar surfaces. To estimate the degree of inhibition of bacterial growth exerted by the crude sponge extract, two replicates of both test concentrations were run and the total width of the inhibition zone for each was recorded. The inhibition value used was the average of the two replicates. This was given a rating on a three point scale, i.e., 1–2 mm = +; 2–3 mm = ++; 3–4 mm = +++. The inhibition zones on the control discs were also noted.

## RESULTS

The results of the agar-plate screening of the two concentrations of sponge extract are given in table 1. It clearly shows that at the 25 µg/disc concentration, antimicrobial activity was restricted to *Vibrio* sp. (+) and *Pseudomonas* sp. (++) (figure 1). At the 50 µg/disc concentration, the extract inhibited growth in all strains of bacteria tested. The activity of the extracts was greatest on the *Vibrio* sp. (+++) and least on *Alteromonas* sp. (+) (figure 1). Except in *Pseudomonas* sp. (where the activity remained unchanged), the antimicrobial activity was higher at the higher concentration.

## DISCUSSION

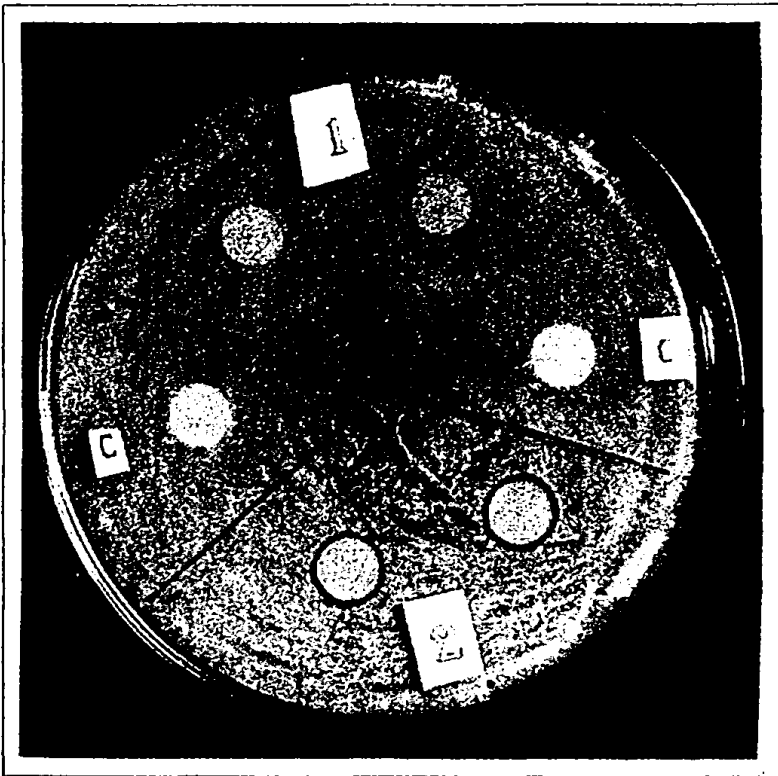
Sponges are a well investigated group in studies related to antimicrobial and antifouling activities. Bergquist and Bedford (1978) reported that

**Table 1.** Antimicrobial activities at two concentrations of crude extracts of *Craniella* sp. against bacterial strains

Sl. No.	Species	Concentration		Control
		25 µg/disc	50 µg/disc	
1.	<i>Vibrio</i> sp.	+	+++	-
2.	<i>Pseudomonas</i> sp.	++	++	-
3.	<i>Escherichia coli</i>	-	++	-
4.	<i>Flavobacterium</i> sp.	-	++	-
5.	<i>Aeromonas</i> sp.	-	++	-
6.	<i>Alteromonas</i> sp.	-	+	-

+ = 1-2 mm, ++ = 2-3 mm, +++ = 3-4 mm, zone of inhibition.

antimicrobial activity exhibited by sponges is more prevalent in temperate than in tropical species. However, they defined Mediterranean species as tropical. It is now felt that the incidence of antimicrobial activity in any particular group is significantly higher in tropical than in temperate- or



**Figure 1.** Antimicrobial activities of crude extracts of *Craniella* sp. against *Vibrio* sp. 1 = 25 µg/disc; 2 = 50 µg/disc, C = control.

Table 2. Comparison of antimicrobial activities against marine bacteria by sponge species belonging to the Choristida group.

Sr. No.	Species	Shape and Location	Concentration (µg)	Bacterial Strain*						Reference
				I	II	III	IV	V	VI	
1.	<i>Sielella maori</i>	spherical, 36°S	30/6 mm	+	-	-	-	-	-	Berquist & Bedford (1978)
2.	<i>Ancornia alata</i>	subspherical, 36°S	30/6 mm	-	+	+	+	+	+	Berquist & Bedford (1978)
3.	<i>Pachymatisma jobynstonii</i>	mass, 48°N	500/6 mm	-	-	-	-	-	-	Amade et al. (1982)
4.	<i>Jaspis stelfifera</i>	globular, 27°N	100/6 mm	-	-	-	-	-	-	McCaffrey & Eudean (1985)
5.	<i>Geodia mesotriaena</i>	spherical, 33°N	-	-	-	-	-	-	-	Thompson et al. (1985)
6.	<i>Penares cortius</i>	mass, 33°N	-	-	-	-	-	-	-	Thompson et al. (1985)
7.	<i>Stellata clarella</i>	mass 33°N	-	-	-	-	-	-	-	Thompson et al. (1985)
8.	<i>Cliona celeta californica</i> **	encrusting, 33°N	-	-	-	-	-	-	-	Thompson et al. (1985)
9.	<i>Craniella</i> sp.	spherical, 16°N	50/7 mm	+	+	+	+	+	+	Present study

\* I = *Vibrio* sp.; II = *E. coli*; III = *Pseudomonas* sp.; IV = *Flavobacterium* sp.; V = *Aeromonas* sp.; VI = *Aeromonas* sp. + = positive; - = negative;

\*\* = doubtful placement in this group.

cold-water members of the same group (Bakus and Kawaguchi 1984). Table 2 gives a comparative account of the antimicrobial activities of some sponge species (belonging to the Choristida group) against marine bacteria. It is clear that only *Stelletta maori* was positive against *Vibrio* sp. and *Ancorina alata* against *E. coli* and *Pseudomonas* sp. The zone of inhibition, at a concentration of 30  $\mu\text{g}/6$  mm disc, was 1-2 mm (+) (Bergquist and Bedford 1978). In that experiment, extraction was carried out with methylene chloride. Other sponge species, even at concentrations of 100 and 500  $\mu\text{g}/6$  mm disc (McCaffrey and Endean 1985, Amade et al. 1982), showed no antimicrobial activity. The *Craniella* sp., however, was positive against all the strains at a concentration of 50  $\mu\text{g}/7$  mm disc. This comparison suggests that antimicrobial activity against marine bacterial strains is more pronounced in tropical species of the Choristida group than in their counterparts from temperate or cold waters. However, detailed studies with uniform methods and techniques need to be conducted to determine this. Further investigations on antifouling and antialgal activities of the extract from *Craniella* species are in progress.

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