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Antimicrobial activity of tropical and subtropical sponges

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Abstract

Extracts of 19 of 24 species of sponge collected from Queensland, Australia, inhibited the growth of test microorganisms in *in-vitro* assays. A similar result has been obtained by earlier workers for extracts of species of sponge obtained from temperate waters. Some of the extracts, including extracts of *Thorecta vasiforis, Arenochalina mirabilis* and *Acanthella kleutha,* showed activity against bacterial strains that was comparable with that exhibited by penicillin G and streptomycin against these strains. Gram-positive bacteria were expecially sensitive to many extracts. Little activity was exhibited by any extract against four species of fungi tested. Some of the extracts were markedly toxic to one or more of the test organisms (a fish, a crustacean and a hydroid) used, but no clear pattern linking toxicity to these organisms with antimicrobial properties of the extracts emerged. In general, there was a negative correlation between antimicrobial activity and surface-fouling, raising the possibility of using freedom from surface-fouling as an indicator of antimicrobial activity. Four of five encrusting species from the undersides of coral boulders showed antimicrobial activity. This study confirmed the value of using methanol-toluene extracts in field-based screening programmes, but emphasised the need to use additional extracting media such as methylene chloride in order to augment the number of antimicrobial compounds detected. There are indications that antimicrobial activity may be widespread in the order Dictyoceratida, in the order Verongida and in the order Axinellida, but sporadic in other orders of Porifera.

Introduction

Marine sponges have provided the natural-products chemist and marine biologist, alike, with a wealth of compounds possessing unusual chemical structures and interesting biological activities. The chemical nature of metabolites isolated from marine sponges has been extensively reviewed by several authors (Burkholder, 1973; Baker and Murphy, 1976, 1981; Minale, 1976; Faulkner, 1977; Wells, 1979). Accounts of the biological activities possessed by these metabolites have been particularly concerned with antimicrobial activity. Faulkner (1978), Shield and Rinehart (1978) and Rinehart *et al.* (1981) noted the chemical structures and activities of a large number of antimicrobial metabolites isolated from sponges. Similarly, Bergquist and Bedford (1978) and Amade (1982) assessed the activities of extracts from New Zealand Demospongiae and sponges from French Polynesia and Brittany, respectively. It was found by Bergquist and Bedford (1978) that antimicrobial activity exhibited by sponges is more prevalent in temperate than in tropical species. This is contrary to the general belief that the incidence of toxicity generally is higher amongst the biota of tropical environments, particularly complex coral-reef environments (Bakus, 1981; Endean and Cameron, 1983). However, Bergquist and Bedford (1978) equated "Mediterranean" species with "tropical" species. This may or may not be justified. It was therefore decided to examine the antimicrobial activities of extracts obtained from a collection of tropical and subtropical sponge species from Pacific waters and to compare these with the activities of temperate sponge species from Pacific waters studied by Bergquist and Bedford.

There is a need for criteria to be established that will facilitate a selection of sponges most likely to exhibit antimicrobial activity. Accordingly, it was decided to ascertain whether antimicrobial activity is confined to particular taxonomic groupings among the species tested, and whether species possessing antimicrobial activity also exhibit external indications of general toxicity such as obvious zones of antibiosis, or a decreased incidence of fouling. In addition, there is a need to develop suitable screening procedures for the rapid testing of sponges for antimicrobial activity at collection sites. Often, antimicrobial activity is an expression of general antibiotic activity.

Accordingly, it was decided to include suitable rapid tests for the detection of general antibiotic activity in the overall screening procedure as well as to include a suitable extraction procedure for the rapid detection of antimicrobial activity at the field site.

Bergquist and Bedford (1978) found that sponge extracts which they used inhibited growth of Gram-negative bacteria markedly, but only a small percentage inhibited Gram-positive bacteria. This result was directly opposed to the findings of Burkholder and Ruetzler (1969), so it was decided to re-examine the situation by including a selection of both Gram-positive and Gram-negative bacteria in the screening procedure to be adopted. At the same time, the opportunity was taken to include tests for anti-fungal activity.

Materials and methods

Collection and extraction

Twenty four species of marine Porifera were collected by hand using SCUBA or snorkel. Common, exposed, non-encrusting specimens were collected from several locations in Moreton Bay (27°25′S; 153°32′E) and from Heron Reef and the adjacent Wistari Reef in the Capricorn Group off Gladstone (23°27′S; 151°53′E). Encrusting sponges were collected from the undersides of coral boulders located on the outer reef crest on Heron Reef. Individuals representative of each species collected were preserved in 70% alcohol and deposited with the Northern Territory Museum, Darwin, for taxonomic identification.

Two methods of extraction were employed in this study. The first method was used for initial field-based evaluation of the antimicrobial activity of the sponges. It involved the preparation of methanol-toluene extracts of freshly collected specimens of each species following a modification of the procedure of Rinehart *et al.* (1981). Two grams of freshtissue were homogenized in 20 ml of methanol-toluene (3:1 v/v), and extracted for 24 h at room temperature. The extracts were centrifuged and the supernatants retained for evaluation of their antimicrobial activity. The second method involved specimens that had been frozen after collection and transported to a mainland laboratory. Here, frozen specimens of each sponge were weighed, lyophilized and powdered. The powdered material was sequentially extracted in methylene chloride and 50% aqueous ethanol. The crude extracts were each concentrated by removal of the solvent under reduced pressure. In the case of the crude aqueous ethanol extract, the aqueous suspension remaining after solvent removal was lyophilized.

Because of the limited quantities of sponge available for collection, only methanol-toluene extracts of the encrusting sponges collected were used.

Antibiotic assays

The general antibiotic activity of the sponges against three different indicator organisms was investigated. Organisms chosen were the mosquito fish *Gambusia affinis,* the first-stage nauplii of the brine shrimp *Artemia* sp., and the athecate hydroid *Solandaria fusca.* The hydroids were collected from the undersides of coral boulders located on the outer reef crest at Heron Reef. Specimens of the hydroids were placed in aerated sea water aquaria and used within one hour of collection. Assays using the mosquito fish and brine shrimp larvae were performed in the Zoology Department, University of Queensland.

Aqueous extracts of the sponge species collected were prepared by homogenizing 20 g of sponge tissue in 40 ml of either distilled water or sea water. The homogenates were centrifuged and the supernatants retained for the assays.

A branch of the hydroid colony containing 10 to 20 individual polyps was placed in a small glass dish containing a 1:20 dilution of the sea water-based extract in sea water. Control branches were placed in sea water alone. The hydroids were observed at 15 min intervals for 60 min, after which time they were transferred to fresh sea water and observed for a further 30 min. Death of the hydroid was established when the polyps fell off the branches, or when they showed marked irreversible swelling.

For assessment of the activity of sponge extracts against *Gambusia affinis* and *Artemia* sp., six adult or sub-adult fish (mean length 1 to 3 cm) and 15 to 20 nauplii were placed separately in glass beakers containing a 1:20 dilution of the distilled water-based extract in distilled water. The numbers of fish or nauplii suffering mortality at 1.5, 3 and 6 h were recorded. Controls were placed in distilled water alone. Each assay was repeated three times.

Because only small quantities of tissue were available, assays involving encrusting sponges were limited to tests against hydroids. (The activities of extracts of sponges collected from Moreton Bay did not include assays involving hydroids.)

Antimicrobial assays

The antimicrobial activity of the sponge extracts was assessed using the disc-assay method (Acar, 1980). Crude methylene chloride and aqueous ethanol extracts were dissolved in aliquots of the extraction solvent and applied to sterile filter-paper discs (6 mm diam) to give a final disc loading of 100 μg. One hundred μl of the methanol-toluene extracts were applied to filter-paper discs of 12.7 mm diam. Discs were placed on agar plates previously seeded with the selected microorganisms. The antibacterial activities of the sponge extracts were compared with those of standard antibiotics penicillin G (10 units) and streptomycin (10 μg). The activities of the control antibiotic compounds against the test bacteria are listed in Table 3.

Sponge extracts were screened for activity against 14 species of microorganisms. These included four Gram-positive and six Gram-negative bacteria and four species of fungi, including two yeasts, a mould and a dermato-

Table 1. Classification and description of the marine sponges screened for antimicrobial activity. +: presence; –: absence

Order, genus, species	Collection site	Description	Cyano-bacteria	Fouling organisms
Dictyoceratida				
Spongia officinalis	Wistari Reef (20 m)	Globular; green ectosome, beige endosome	+	–
Spongionella sp.	Moreton Bay (15 m)	Cylindrical branches from a stalked base; black	–	–
Fascaplysinopsis reticulata	Moreton Bay (12 m)	Globular; green ectosome, yellow endosome	+	–
Dysidea herbacea	Lagoon, Heron Island (3 m)	Thin cylindrical branches; green	+	–
Dysidea sp. 1	Reef crest, Heron Island	Encrusting	–	–
Dysidea sp. 2	Reef crest, Heron Island	Encrusting	–	–
Thorecta vasiforis	Wistari Reef (30 m)	Large vase-shaped sponge; grey-purple	–	–
Ircinia sp.	Hervey Bay (3 m)	Foliaceous branches; olive	+	+
Poecilosclerida				
Arenochalina mirabilis	Wistari Reef (20 m)	Erect tubular branches from an encrusting base; purple	–	–
Neofibularia irata	Wistari Reef (20 m)	Plate-like branches from a basal spreading mat; red/brown	+	–
Psammopemma sp. 1	Wistari Reef (15 m)	Thin cylindrical branches; orange	–	–
Psammopemma sp. 2	Moreton Bay (12 m)	Tubular branches; purple	–	+
Iotrochota coccinea	Moreton Bay (3 m)	Encrusting; purple	–	–
Haplosclerida				
Euplacella sp. 1	Moreton Bay (15 m)	Erect lamellae; green	–	–
Euplacella sp. 2	Moreton Bay (7 m)	Erect tubular branches; purple	–	–
Callyspongia sp.	Moreton Bay (7 m)	Erect, spreading; beige	–	–
Callyspongia muricinia	Wistari Reef (20 m)	Erect, spreading; white	–	–
Spinosella sp. 1	Reef crest, Heron Island	Encrusting; blue	–	–
Spinosella sp. 2	Reef crest, Heron Island	Encrusting; yellow	–	–
Adocia sp.	Hervey Bay (4 m)	Erect tubular branches; purple	–	+
Choristida				
Jaspis stellifera	Reef flat, Heron Island	Globular; brown ectosome, yellow endosome	+	+
Spirophorida				
Raphiotethya enigmatica	Wistari Reef (20 m)	Globular; pink	–	–
Axinellida				
Acanthella kleutha	Wistari Reef	Globular; orange	+	–
Dendroceratida				
Aplysilla sp.	Reef crest, Heron Island	Encrusting; red	–	–

phyte. Three of the six Gram-negative bacteria were marine strains. The non-marine microorganisms and the marine bacterium *Pseudomonas putida* were obtained from the Department of Microbiology, University of Queensland. The marine strains designated S1 and S2 were isolated from sea water sampled from a depth of 10 m in the channel between Heron Reef and Wistari Reef. Repeated sub-culturing of colonies from cultures maintained on sea water nutrient-agar yielded Strains S1 and S2. Both are gram-negative coccoid strains, S1 being 0.7 μm in diameter, and S2 being an encapsulated bacterium, 0.3 μm in diameter. These strains have not been further characterized to date. The non-marine bacteria were maintained on Muellar-Hinton nutrient-agar plates (Gibco) at pH 7.3. Zones of inhibition, if any, were measured following overnight incubation of the plates at 37 °C. The three marine bacteria were maintained on sea water nutrient-agar plates, and zones of inhibition were measured following incubation at 28 °C over 48 h.

Suspensions of the yeasts *Candida albicans* and *Saccharomyces cerevisiae* were prepared in Sabouraud liquid medium (Oxoid) and maintained on Sabouraud dextrose-agar plates (Oxoid). Suspensions of the mould *Aspergillus fumigatis* and the dermatophyte *Tricophyton mentagrophytes* were grown in Sabouraud liquid medium. The cultures were homogenized and diluted with Sabouraud liquid medium to give a final concentration of 1×10^4 cells ml^{-1}. One ml of the fungal suspensions was spread onto Sabouraud dextrose-agar plates, and zones of inhibition were measured following incubation at 28 °C. The period of incubation ranged from 48 h for the yeasts to 96 h for the filamentous fungi.

Results and discussion

The classification, site of collection and characteristic features of each sponge species studied are noted in Table 1. This assemblage of sponges constitutes a representative selection of the commoner tropical and sub-tropical species encountered at the collection sites. However, the rarer species present at these sites are not well represented

Table 2. Inhibition of bacterial and fungal growth by sponge extracts. A: MeOH-toluene 3:1 v/v; B: methylene chloride; C: 50% aqueous ethanol. –: no activity; +: < 2 mm; ++: 2 to 5 mm; +++: 5 to 12 mm; ++++: > 12 mm

Sponge species and extract	Gram$^+$ bacteria				Gram$^-$ bacteria				Fungi					
	Staphylococcus aureus	*Staphylococcus epidermidus*	*Bacillus subtilis*	*Streptococcus lactis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas putida*	S1	S2	*Candida albicans*	*Saccharomyces cerevisiae*	*Aspergillus fumigatis*	*Tricophyton menatagrophytes*
Thorecta vasiforis														
A	++++	–	+++	++	–	–	–	–	–	–	–	–	–	–
B	++	–	++	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spongia officinalis														
A	–	+++	++	+++	–	–	–	–	–	–	–	–	–	–
B	–	–	++	++	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spongionella sp.														
A	–	–	–	++	–	–	–	++	–	–	–	–	–	–
B	–	–	+	+	+	–	–	++	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fascaplysinopsis reticulata														
A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	–	++	++	++	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dysidea herbacea														
A	+++	–	+	+++	–	–	–	–	–	–	–	+	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	++	–	–	–	–	–	–	–	–
Dysidea sp. 1														
A	++	++	++	++	–	–	–	–	–	–	–	–	–	–
Dysidea sp. 2														
A	++	–	–	+++	–	–	–	–	–	–	–	–	–	–
Ircinia sp.														
A	–	–	+	++	–	–	–	–	+	+	–	–	–	–
B	++	–	++	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Arenochalina mirabilis														
A	+++	–	+++	++	–	–	–	–	–	+	+	–	–	+
B	++++	+	+++	++	–	–	–	+	–	–	–	–	–	+
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Neofibularia irata														
A	–	–	–	–	–	–	–	–	–	+	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Psammopemma sp. 1														
A	–	–	–	++	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Psammopemma sp. 2														
A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	+++	–	–	–	+	++	–	–	–	–	–	+	–	–
C	+++	–	–	–	–	–	–	–	–	–	–	+	–	–
Iotrochota coccinea														
A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	+	++	–	–	–	–	–	–	–	–	++	–	–	–
C	++	–	++	–	–	–	–	–	–	–	–	–	–	–
Euplacella sp. 1														
A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 2 (continued)

Sponge species and extract	Gram⁺ bacteria				Gram⁻ bacteria				Fungi					
	Staphylococcus aureus	*Staphylococcus epidermidus*	*Bacillus subtilis*	*Streptococcus lactis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas putida*	S1	S2	*Candida albicans*	*Saccharomyces cerevisiae*	*Aspergillus fumigatis*	*Tricophyton menatagrophytes*
Euplacella sp. 2														
A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	–	+	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Callyspongia sp.														
A	–	–	–	+ +	–	–	–	–	–	+	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Callyspongia muricinia														
A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spinosella sp. 1														
A	+ + +	+ + + +	+ + + +	+ + + +	–	+ +	–	–	–	–	–	–	–	–
Spinosella sp. 2														
A	+ +	–	+ + +	+ + +	–	–	–	–	–	–	–	–	–	–
Adocia sp.														
A	–	–	–	–	–	–	–	–	+	+	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Jaspis stellifera														
A	–	+ + +	–	–	–	–	–	–	–	+	–	–	–	–
B	–	+ +	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Raphiotethya enigmatica														
A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Acanthella kleutha														
A	+	–	+ + +	–	–	–	+	–	–	–	–	–	–	–
B	+ + + +	+ + +	+ + +	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aplysilla sp.														
A	+	–	–	–	–	–	–	–	–	–	–	–	–	–

in the collection. They will be the subject of a subsequent collection programme. Only members of the class Demospongiae were, in fact, observed at the collection sites. Of this class, the orders Dictyoceratida, Poecilosclerida and Haplosclerida were best represented. Unfortunately, the taxonomy of tropical and sub-tropical sponges is poorly known and, in several cases, it has not been possible to identify the species collected. These species have been catalogued and categorised for field recognition and further attempts to identify them are being made.

The results of the antimicrobial screening of the sponge extracts and the standard antibiotics are listed in Tables 2 and 3. Table 2 shows that 19 of the 24 species assayed (79.2%) inhibited the growth of one or more of the micro-organisms tested. This result is similar to that obtained for temperate sponge species from New Zealand, where 87% of the species assayed showed some inhibitory activity (Bergquist and Bedford, 1978).

Because relatively few representatives of major taxonomic groups of the Porifera were included in this study, it is not possible to draw any far-reaching conclusions about the relationship between taxonomic position and the extent of antimicrobial activity. However, the available evidence from this and earlier studies indicates that

members of the order Dictyoceratida, order Verongida and order Axinellida usually exhibit antimicrobial activity, but that such activity appears to be sporadic in other orders.

Although little activity was exhibited by any extract against the four species of fungi tested, potent antibacterial activity was exhibited by many of the extracts. In particular, the methanol-toluene extracts of *Thorecta vasiforis* and *Spinosella* sp. 1 and the methylene chloride extracts of *Arenochalina mirabilis* and *Acanthella kleutha* showed potent activity against several of the Gram-positive bacteria. In these cases, the activity exhibited was comparable with that exhibited by the standard antibiotics penicillin G and streptomycin against these strains. The lack of activity against the four species of fungi may reflect the insensitivity of the disc-assay method for assessing anti-fungal properties. The use of broth and/or agar dilution tests may improve the sensitivity of the screening procedure.

The results of the screening for general antibiotic activity of the aqueous extracts of the sponges collected against test organisms are listed in Table 4. Some of the extracts were markedly toxic to one or more of the test organisms, but no clear pattern linking antimicrobial and antibiotic properties for this collection of sponges emerged from these experiments. Sponges found to possess strong antimicrobial properties usually showed a high level of toxic activity against the indicator organisms. However, this was not always so. For example, *Euplacella* sp. 1 and *Callyspongia muricinia* were both highly toxic to the test organisms (Table 4), yet displayed no antimicrobial activities in these studies (Table 2). Further work using a variety of extracting media is required before a decision can be made on the value of using organisms such as fish, brine shrimps and hydroids in preliminary screening programmes for antimicrobial compounds.

Nakutsu *et al.* (1983) noted a negative correlation between the presence of antimicrobial activity and the amount of surface-fouling displayed by sponges, suggesting a possible antifouling role for the active constituents. A similar hypothesis was proposed by Burkholder (1973) to account for the lack of surface-fouling in gorgonians. In accordance with these ideas, sponges found to possess antimicrobial properties in this study were generally free of fouling organisms. However, several species were exceptions to this trend. Both *Psammopemma* sp. 2 and *Jaspis stellifera* showed relatively heavy fouling although possessing some antimicrobial properties, whereas both species of *Euplacella* screened displayed no antimicrobial activity, although they were especially free of epibiotic growth.

It has been reported that some sponge metabolites possess a range of biological activities, including antimicrobial properties. These findings have led to speculation as to the possible ecological significance of these compounds. The antimicrobial constituents of the Pacific sponge *Agelas* sp. (Capon and Faulkner, 1984) and of *Toxadocia zumi* (Nakutsu *et al.*, 1983) have been reported to be ichthyotoxic and to prevent the division of fertilized sea-urchin eggs. Sullivan *et al.* (1981) reported the isolation of two antimicrobial metabolites from the coral-burrowing sponge *Siphonodictyon coralliphagum*. It was

Table 3. Activity of control antibiotics penicillin G (Mastring polydisc 309) and streptomycin (Mastring polydisc 243) against test bacteria. Activity is expressed as the radius of the zone of inhibition of bacterial growth, in mm. –: no inhibition; +: < 2 mm; + +: 2 to 5 mm; + + +: 5 to 12 mm; + + + +: > 12 mm

Bacteria	Penicillin G (10 units)	Streptomycin (10 mg)
Staphylococcus aureus	+ + +	+ +
Staphylococcus epidermidis	+ +	+ + +
Bacillus subtilis	+ +	+ + +
Streptococcus lactis	+ + +	+ + +
Pseudomonas aeruginosa	–	–
Escherichia coli	–	+ + +
Klebsiella pneumoniae	–	+ + + +
Pseudomonas putida	–	–
S1	–	+
S2	–	+

Table 4. Antibiotic activity of aqueous sponge extracts. Results are expressed as percentage of test organisms dead at end of the observation period. nt: assay not performed

Sponge species	Test organisms		
	Gambusia affinis	*Artemia* sp.	*Solandaria fusca*
Dictyoceratida			
Spongia officinalis	50	0	30
Spongionella sp.	0	0	nt
Fascaplysinopsis reticulata	33	20	nt
Dysidea herbacea	87	100	100
Dysidea sp. 1	nt	nt	20
Dysidea sp. 2	nt	nt	20
Thorecta vasiforis	87	60	100
Ircinia sp.	40	20	nt
Poecilosclerida			
Arenochalina mirabilis	50	20	40
Neofibularia irata	0	20	20
Psammopemma sp. 1	33	0	nt
Psammopemma sp. 2	15	0	0
Iotrochota coccinea	67	30	nt
Haplosclerida			
Euplacella sp. 1	67	100	nt
Euplacella sp. 2	33	0	0
Callyspongia sp.	15	0	nt
Callyspongia muricinia	100	70	100
Spinosella sp. 1	nt	nt	100
Spinosella sp. 2	nt	nt	100
Adocia sp.	0	0	0
Choristida			
Jaspis stellifera	10	70	50
Spirophorida			
Raphiotethya enigmatica	20	0	10
Axinellida			
Acanthella kleutha	100	10	100
Dendroceratida			
Aplysilla sp.	nt	nt	40

suggested that these compounds may play a role in the burrowing mechanism of the sponge, or in the maintenance of a dead zone between the sponge and the growing edge of adjacent coral. These examples lend support to the hypotheses of Green (1977) and Bakus (1981) that bioactive compounds are of importance in determining the interactions of sponges with other organisms within their environment. However, the influence of confirmed antimicrobial metabolites on interactions other than those involving microorganisms remains unclear. It is an area of study that warrants further investigation.

In this context, it was interesting to note that four of five species collected from the undersides of coral boulders showed activity against a number of the test organisms. These sponges were amongst the dominant organisms of these communities, with the two species of *Spinosella* collected observed to be overgrowing associated colonial ascidians and bryozoans (own unpublished results). If the active compounds possess bioactivities other than antimicrobial properties, it is conceivable that these compounds may aid in procuring and maintaining living space in a community system where space is regarded as a limiting factor (Jackson and Buss, 1975). None of the exposed sponges collected were observed to be overgrowing other species at the time of collection, or to be associated with any obvious zones of antibiosis. However, the release of allelochemicals by sponges may be intermittent. The possibility must be entertained that the antibiotic substances present in the extracts are artefacts of the extraction processes used, and have no antimicrobial or antibiotic activity in the marine ecosystem.

Gram-positive bacteria were found in the present study to be particularly sensitive to the sponge extracts, whilst very little activity was recorded against the Gram-negative strains, including the marine bacteria. This result differs from those obtained by Bergquist and Bedford (1978) and Amade *et al.* (1982). In their study of the incidence of antibacterial activity in New Zealand Demospongiae, Bergquist and Bedford found Gram-negative strains to be more susceptible to the effects of sponge extracts than were Gram-positive bacteria. Indeed, these results led them to propose that the active constituents in the sponges act to increase the efficiency of the capture and digestion of particulate organic matter by causing a mild clumping of susceptible bacteria and, hence, increasing the size of particles trapped at the choanocyte chambers. If this system represents an integral part of the feeding mechanism of marine Demospongiae, it would be expected that other groups of sponges of this taxon would consistently exhibit a high level of antibacterial activity against Gram-negative bacteria. This has not proved to be the case. Indeed, a reversal of this trend was recorded in this study as well as in an earlier study by Burkholder and Ruetzler (1969). It therefore seems unlikely that the role of the antimicrobial constituents of this group of sponges can be rationalized in terms of the proposal of Bergquist and Bedford.

The assigning of "ecological roles" to marine natural products is complicated by the nature of the symbiotic relationships observed in many marine invertebrates, including sponges. Symbiotic organisms associated with sponges have been identified as the sources of bioactive metabolites including acanthifolicin from *Pandaros acanthifolium* (Schmitz *et al.*, 1981), okadaic acid from *Halichondria okadi* and *H. melanodocia* (Tachibana *et al.*, 1981) and the many and varied metabolites isolated from *Dysidea herbacea* (Carte and Faulkner, 1981; Erickson and Wells, 1982; Kazlauskas *et al.*, 1978). It is possible that the ecological significance attributed to antimicrobial metabolites associated with a particular sponge may be incidental, dependent upon the presence and metabolic processes of a symbiotic organism. Sponges found to possess blue/green algal symbionts in this study are indicated in Table 1. The role, if any, of these symbionts in the production of antimicrobial metabolites in the host sponge has yet to be determined.

Sixteen of the 24 species studied in this work exhibited positive antimicrobial activity in field-based screening of the methanol-toluene extracts and, genereally, sponges active in this screening showed activity in one or both of the other extracts employed. The use of this type of primary screening for evaluating the presence of bioactive compounds in marine flora and fauna has been advocated by Rinehart *et al.* (1981). These workers assessed the cytotoxic and antimicrobial activity of the methanol-toluene extracts of over 1 000 marine species in expeditions to Baja California and the Caribbean Sea. A programme of this type offers several advantages to marine scientists with an interest in marine bioactive compounds. In particular, the programme is amenable to situations where access to laboratory test-animals and equipment is often limited; e.g. small research vessels and field stations. Also, only small quantities of each organism are required for the assays, thereby ensuring that disturbance to the environment is minimal.

However, there does exist the seemingly unavoidable problem that compounds possessing potent bioactivities will be overlooked, especially if the selection of species for re-collection and further study is made solely upon the results of an initial field-based screening. This problem was encountered to some degree during the course of this study, several of the species collected being found to possess antimicrobial properties that were not evident in the primary screening. Activity expressed by extracts of the sponge *Acanthella kleutha* illustrates this finding. The methanol-toluene extract was only mildly effective against *Staphylococcus aureus,* whereas the methylene chloride extract potently inhibited the growth of both *S. aureus* and *S. epidermidis.* Despite such anomalies, the large number of species found to possess bioactive substances as the result of field-based screenings using methanol-toluene extracts attests to its usefulness. It is difficult to envisage ways of increasing the efficiency of this method without reducing its essential simplicity and flexibility.

The results of the present study have shown that extracts of many Australian tropical and sub-tropical sponges certainly possess potent *in-vitro* antimicrobial

activities, particularly against Gram-positive bacteria. Some of the active constituents are now being evaluated as potential clinical agents.

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