(*Reprinted from Nature, Vol. 222, No. 5197, pp. 983–984, June 7, 1969*)

Antimicrobial Activity of some Marine Sponges*

THE evaluation of marine plants and animals for potential use in chemotherapy poses problems of procurement of materials and of techniques of screening for significant drug activity[1]. We have been studying antimicrobial activity in marine sponges both from the viewpoint of marine ecology and in an effort to develop new drugs, and report on our methods and some of the results obtained with collections from the Caribbean Sea, Mediterranean Sea and the Pacific Ocean.

We collected marine invertebrates by scuba diving in shallow waters to depths of about 30 m. The animals were either frozen at low temperature or dehydrated by gentle heating. For systematic study additional specimens were fixed in 10 per cent formalin in seawater and preserved in 70 per cent ethanol. A collection of forty-one Mediterranean sponges consisted of small samples, each comprising about 1 g wet weight. The samples were freeze dried and transported by air to the laboratory at Lamont Geological Observatory, where they were screened for antimicrobial activity. The dried specimens were ground to a powder with a glass mortar and pestle and then moistened with citrate–phosphate buffer adjusted to *p*H 7·0. Small portions of the resultant slurry were transferred on one-quarter inch filter paper disks to the seeded 'Difco' nutrient agar test plates. For *Candida*, 2 per cent glucose was added to the medium. Incubation was at 32° C overnight. Nutrient seawater agar was used for all the marine bacteria. Results of tests for activity of Mediterranean sponges against five microorganisms are shown in Table 1. Of thirty-one species of sponge, eighteen showed antimicrobial activity against one or more of the test organisms. The marine bacterium B-746 showed greatest susceptibility to growth inhibitors of these sponges, and *Candida albicans* exhibited the least. Some of the sponges, for example, *Verongia aerophoba*, *Crambe crambe* and *Aplysilla sulfurea*, apparently contain substances capable of inhibiting both Gram positive and negative bacteria.

* Contribution No. 1269.

Table 1. ANTIMICROBIAL ACTIVITY OF SPONGES FROM THE MEDITERRANEAN SEA

Sponge species	*Bacillus subtilis*	*Escherichia coli*	*Candida albicans*	*Mycobacterium phlei*	Marine 746
Verongia aerophoba (O. Schm.)	15	11	0	12	13
Crambe crambe (O. Schm.)	3	3	3	6	4
Aplysilla sulfurea F. E. Schulze	4 partial	3	0	6	5
Spongia virgultosa (O. Schm.)	4	0	0	4	7
Ircinia variabilis O. Schm.	4 partial	0	0	4	3·5
Hemimycale columella (Bow.)	2	2	0	4 partial	3·5
Cliona carteri (Ridl.)	0	1	0	5 partial	3
Anchinoe ficticius (Bow)	3 partial	0	0	3 partial	2
Spirastrella cunctatrix O. Schm.	0	0	0	4	3·5
Ircinia fasciculata (Pallas)	2	0	0	3 partial	2
Petrosia ficiformis (Poiret)	0	0	0	4	2·5
Placospongia decorticans (Hanitsch)	0	2	0	0	4
Cliona copiosa Sará	6 partial	0	0	0	0
Fasciospongia cavernosa (O. Schm.)	3·5	0	0	0	0
Pellina semitubulosa (Lieberk.)	0	0	0	3	0
Clathria toxistyla (Sará)	0	0	0	0	2·5
Crella rosea (Tops.)	0	0	0	0	2
Geodia cydonium (Jam.)	0	0	0	0	2

Values are expressed in mm radius.

The ecological significance of inhibition and promotion of microbial growth by sponge extracts is illustrated in these simple experiments with laboratory and marine cultures of bacteria grown in 'Difco' nutrient agar. Although the nutrient medium allows good growth of common strains of bacteria, nearly all of the sponge slurries produced greater growth zones around the paper disks or outside the areas of antibiosis in the culture plates of *B. subtilis*, *E. coli* and the marine bacterium B-746. This marine organism requires thiamine, biotin and either vitamin B_{12} or methionine, and appears to bo very sensitive to biologically active substances. Although all these sponges exhibited growth promotion, some failed to show any inhibition. Non-antibiotic sponges were: *Ircinia oros* (O. Schm.), *Ircinia* (*Sarcotragus*) *spinosula* (O. Schm.), *Gellius fibulatus* (O. Schm.), *Hymedesmia versicolor* Tops., *Suberites domuncula* (Olivi), *Cliona viridis* (O. Schm.), *Tethya aurantium* (Pallas), *Erylus discoporus* (O. Schm.), *Pachastrella monolifera* (O. Schm.), *Chondrilla nucula* (O. Schm.), *Leuconia solida* (O. Schm.), *Leucosolenia complicata* (Mont.) and *Clathrina blanca* (Mikl.–Maclay). In those species where antimicrobial activity was weak, not all specimens produced zones of inhibition.

Another group of antibiotic sponges selected from shallow waters around Puerto Rico was collected and kept frozen until tests against marine bacteria could be made. Cultures of bacteria typical of the nine computer groups sorted in a numerical taxonomy program[2] were used

Table 2. INHIBITION OF MARINE BACTERIA BY PUERTO RICAN SPONGES

Marine bacteria tested			Number of sponges inhibiting each bacterium
Group	Isolate	Origin	
I	151	S. Georgia	9
II	156	S. Georgia	6
III	170	S. Orkney	11
IV	260	Puerto Rico	10
V	171	S. Georgia	6
VI	219	S. Orkney	8
VII	247	S. Orkney	8
VIII	195	S. Pacific	4
IX	176	S. Pacific	6
	Sa 123	Sapelo Is.	12
	WH 302	Woods Hole	15

Twenty sponges were tested against eleven bacteria, and the zones of inhibition varied from 0 to 60 mm radius.

as indicators of sponge antimicrobial activity. Two additional marine strains, Sa 123 requiring biotin and thiamine, and WH 302 requiring cyanocobalamine, were included because of their apparent sensitivity to sponge antibiotics. The results of this survey, shown in Table 2, indicate that marine bacteria isolated from widely different geographic areas (for example, Woods Hole, Massachusetts; Sapelo Island, Georgia; Puerto Rico and South Orkney Islands) were inhibited by extracts of many sponges. Some bacteria are more susceptible than others, and the number of sponges inhibiting growth of each bacterium was in the range from four to fifteen out of a total of twenty sponges tested. Among the most active sponges were species of *Verongia*.

Comparative studies of antimicrobial activity of marine animals collected in the Great Barrier Reef of Australia and in the Caribbean Sea have been made with essentially the same methods, except that the assays were performed with fresh material. A summary of the screening results is shown in Table 3. About the same percentages of animals in the two oceans are found to be active against the three types of microbial indicators shown in Table 3, although the species of animals are different in the two widely separated geographic regions. In both oceans, more animal extracts inhibit Gram-positive than Gram-negative bacteria or yeast. Some of the active animals provide antimicrobial substances that can be isolated, characterized and synthesized[3]. Articles dealing with the results of further work on isolation and characterization of some new compounds are in preparation.

Table 3. RESULTS OF SCREENING MARINE ANIMALS (CHIEFLY SPONGES) FROM THE PACIFIC AND ATLANTIC OCEANS FOR ANTIMICROBIAL ACTIVITY

Region	Number of samples	Antimicrobial activity		
		Gram +	Gram −	*Candida* sp.
Atlantic Ocean (Caribbean)	777	35%	15%	10%
Pacific Ocean (Great Barrier Reef)	464	39%	12%	11%

This work was supported by a US National Institutes of Health grant and by the G. Unger Vetlesen Foundation.

PAUL R. BURKHOLDER*
Lamont Geological Observatory of
Columbia University,
Palisades, New York.

KLAUS RUETZLER
Smithsonian Institution.
Washington DC.

Received November 18, 1968; revised February 18, 1969.

* Present address: Department of Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico.

[1] Marderosian, A. der, *J. Ocean. Tech.*, 19 (Marine Technology Society, Washington, DC, 1968).
[2] Pfister, R. M., and Burkholder, P. R., *J. Bact.*, **90**, 863 (1965).
[3] Sharma, G. M., Vig, B., and Burkholder, P. R., *J. Ocean. Tech.*, 119 (Marine Technology Society, Washington DC, 1968).

Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.