Applied and Environmental Microbiology, Dec. 1987, p. 2957–2962
0099-2240/87/122957-06$02.00/0

Vol. 53, No. 12

Distribution and Significance of Heterotrophic Marine Bacteria with Antibacterial Activity

SHANTA NAIR†* AND USIO SIMIDU

Ocean Research Institute, University of Tokyo, Nakano-ku, Tokyo 164, Japan

Received 22 June 1987/Accepted 18 September 1987

Bacteria with antibacterial activity were isolated from seawater, sediments, phytoplankton, and zooplankton of Suruga, Sagami, and Tokyo Bays and from soft corals and sponges collected from the Taiwan coast. Of the 726 strains isolated, 37 showed antibacterial activity against either *Vibrio parahaemolyticus* (ATCC 17802) or *Staphylococcus aureus* (P209). Sediment harbored the lowest number of these forms of bacteria, and those from Tokyo Bay did not show any activity. Attached isolates showed greater activity compared with free-living forms. Relatively high numbers of strains with antibacterial activity were associated with phytoplankton. Among the zooplankton isolates, cladocerans harbored the maximum number of antibacterial strains. Isolates were more inhibitory to gram-positive test cultures. Autoinhibition was observed only among 8% of the isolates. Marine nonproducers were more susceptible. *Pseudomonas/Alteromonas* species made up 81.0% of isolates, of which 30% were pigmented strains. The absence or reduction in number of bacteria with antibacterial activity in Tokyo Bay is attributed to its eutrophic nature, which may tend to moderate the production of antibacterial compounds.

Many marine heterotrophic bacteria are known to produce antibacterial substances which inhibit or kill other bacteria. Recent studies have shown that these antibacterial compounds are not only inhibitory to terrestrial bacteria but also to indigenous bacterial strains, which is of considerable ecological significance (17, 18). Isolation and characterization of antibiotic components have been carried out by various researchers (2, 3). Few reports are available on the geographic distribution of these bacteria (2, 12, 16). The ecological amplifications of antibiotic interactions in marine waters remain obscure.

In the present paper, the general distributions of antibacterial strains, obtained from various sources, such as water, sediment, plankton, sponges, and corals from three environments (Tokyo, Sagami, and Suruga Bays) located around Tokyo, are presented.

MATERIALS AND METHODS

Sea water samples from Tokyo, Sagami, and Suruga Bays (Fig. 1) were collected during the K.T. 84-7 cruise of R.V. *Tanseimaru* with Niskin bacteriological water samplers. Sediment samples from these stations were collected with a Phleger-type gravity corer. Zooplankton and phytoplankton were collected with G-54 and XX-13 nets (Rigosha & Co. Ltd., Japan), respectively. Sponges and soft corals were obtained from the southern coast of Taiwan.

Bacterial strains from Tokyo Bay were isolated on spread plates, and colonies from Sagami and Suruga Bays were isolated on Nuclepore filters. Double filtration units with 5- and 0.2-μm-pore-size filters were used, and those bacteria retained on 5-μm-pore-size filters were referred to as attached bacteria, whereas those retained on 0.2-μm-pore-size filters were considered free-living bacteria. For sediment samples, 5 ml of the slurry was suspended in 45 ml of prefiltered seawater. Enumeration and isolation were carried out on ORI plates (20). Zooplankton samples were homogenized in 1 ml of seawater, and 0.1-ml aliquots were spread on ORI medium. Phytoplankton samples were allowed to settle and then agitated with sterile seawater in a mortar. The plates were incubated at 20°C for 10 days. About 726 strains were isolated from various sources and locations and were purified and tested for antibacterial activity by the diffusion agar method used for antibiotic assays. The test strains used were *Vibrio parahaemolyticus* (ATCC 17802) and *Staphylococcus aureus* (P209). The positive strains were tested for their activities against 13 type cultures and a few non-antibiotic-producing isolates to estimate their spectrum of activity. The isolates were identified on the basis of the Simidu scheme (21).

RESULTS

The percentages of strains from various sources which produced antibacterial substances are presented in Table 1. The highest percentage of strains showing antibacterial activity was observed in phytoplankton isolates, and the minimum was observed in those obtained from sponges. Among the total gram-positive strains, however, the percentage was highest for antivibrio activity in the zooplankton isolates and antistaphylococcus activity in the phytoplankton isolates. In the water samples, isolates with more antibacterial activity were obtained from the attached as opposed to the free-living forms. Sediment harbored the lowest percentage of antibacterial strains.

Among the three bays, the isolates from Tokyo Bay did not show antibacterial activity, except for 5% antistaphylococcus activity in strains from phytoplankton (Table 2). No antivibrio activity was noted in free-living strains isolated from the Suruga Bay water. However, antistaphylococcus activity was observed in 2.3% of the strains. The frequencies of isolates scoring positive from free-living population of Sagami Bay were generally higher; however, it was less than the values obtained from attached bacterial population of Sagami and Suruga Bays. All bacterial strains isolated from Tokyo Bay sediments failed to show antibacterial activity. Similarly, those from Sagami Bay did not

* Corresponding author.
† Present address: National Institute of Oceanography, Dona Paula, Goa 403 004, India.

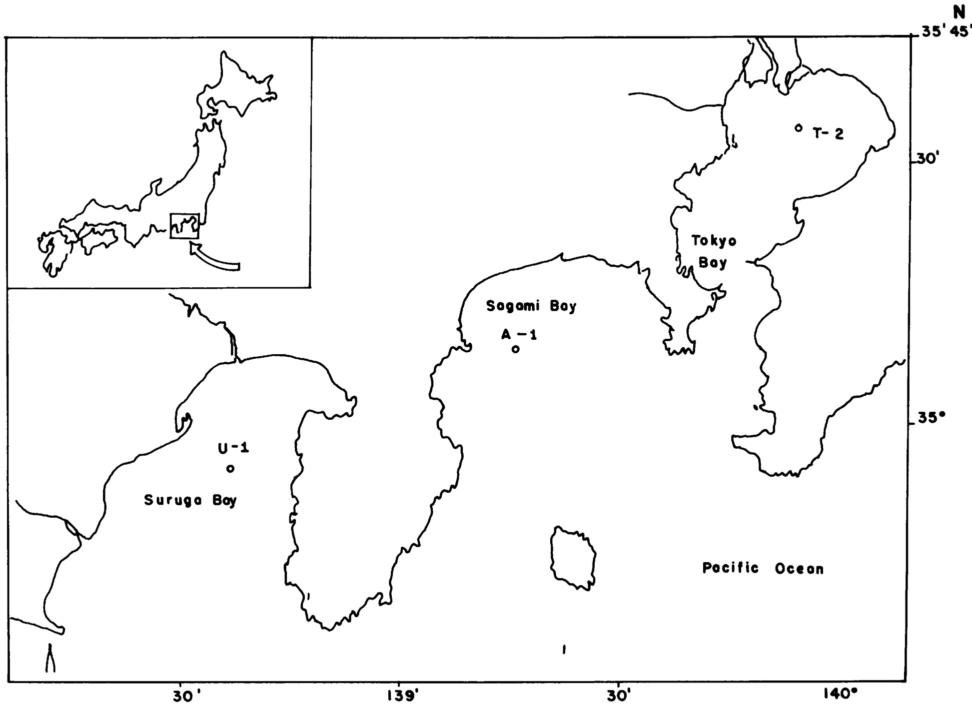

FIG. 1. Location of sampling stations.

exhibit any antistaphylococcus properties, although 1 strain of the 19 strains tested did exhibit antivibrio activity (Table 2).

Among the different groups of zooplankton, the maximum percentage of strains exhibiting antivibrio and antistaphylococcus activities was obtained from cladocerans, followed by chaetognaths, whereas the isolates from decapods did not show any antibacterial activity (Table 3). In general, the frequency of occurrence of isolates with antivibrio activity was higher among the various groups (35.3%; Table 3).

The spectrum of antibacterial activity of the 37 producer strains (strains producing antibacterial compounds) tended to vary from strain to strain. Among the producer strains, two strains belonging to the *Pseudomonas*/*Alteromonas* genus inhibited all 13 type test cultures (Table 4). However, the activities were not uniform even among the species of the genus *Pseudomonas*, i.e., the activity differed even among the different strains of *Pseudomonas*. In general, gram-positive bacteria were more susceptible against producer strains. Most of the pigmented strains showed narrow spectra of activity. Autoinhibition was observed in three strains (8.0%). Comparison of the spectra of producers against producers and against nonproducers (strains that did not produce antibacterial compounds) showed that the latter had higher susceptibility to inhibitory substances (Table 5 and Fig. 2). In contrast, strains isolated from phytoplankton of Sagami Bay had no activity against nonproducer strains. Interestingly, those strains which showed higher spectra of activity against nonproducers had narrow ranges of inhibition with other producers and relatively higher antagonism against type cultures tested.

The most dominant group of antibacterial strains consisted of the *Pseudomonas*/*Alteromonas* isolates (81.0%) (Table 6). *Vibrio* isolates contributed 16.3%. Among the 37 producer strains obtained, 30% were pigmented and only one was a

TABLE 1. Percentage of bacterial strains exhibiting antibacterial activity isolated from different sources

Source (*n*)	% (no.) of antibacterial strains		Total %	
	Antivibrio	Antistaphylococcus	Antivibrio	Antistaphylococcus
Water				
Free (126)	2.4	3.2	12.5	13.3
Attached (116)	6.0	6.9	29.1	26.7
Sediment (39)	2.7	0.0	4.2	0.0
Phytoplankton (40)	10.0	25.0	16.7	33.3
Zooplankton (262)	3.1	2.7	33.3	23.3
Sponges (143)	0.7	0.7	4.2	3.3
Total (726)	3.3 (24)	4.1 (30)	100	100

TABLE 2. Number and percentage of antibacterial strians obtained from different locations

Source (*n*)	% of antibacterial strains	
	Antivibrio	Antistaphylococcus
Water		
Tokyo (35)	0.0	0.0
Free		
Sagami (47)	6.4	6.4
Suruga (44)	0.0	2.3
Tokyo (28)	0.0	0.0
Attached		
Sagami (45)	6.7	8.8
Suruga (43)	9.3	9.3
Tokyo (20)	0.0	0.0
Sediment		
Sagami (19)	5.3	0.0
Tokyo (20)	0.0	5.0
Phytoplankton		
Sagami (20)	20.0	45.0

TABLE 3. Antibacterial activity obtained from different groups of zooplankton at the Aburatsubo Inlet

Group (*n*)	% of antibacterial strains	
	Antivibrio	Antistaphylococcus
Zooplankton (22)	4.5	9.1
Copepod (19)	0.0	5.3
Decapod (19)	0.0	0.0
Cladoceran (19)	10.5	10.5
Chaetognath (20)	10.0	5.0
Total (99)	6.1	6.1

Flavobacterium isolate; the rest were *Pseudomonas* or *Alteromonas* isolates.

DISCUSSION

Of a total of 726 isolates tested, 24 strains inhibited *Vibrio parahaemolyticus*, and 30 strains inhibited *Staphylococcus aureus*. Krasilnokova (11) tested 326 isolates of marine bacteria from a worldwide study and found that 38% showed some degree of inhibition against laboratory strains. When the environments of Tokyo and Sagami Bays were compared, it was observed that 100% of antivibrio strains and 94.1% of antistaphylococcus strains were obtained from Sagami Bay. Okami (14) found that among the isolates from both Japan sea and Pacific Oceans, only a small percentage of bacteria had antibacterial property, whereas more than 20% of the actinomycetes showed antibiosis. Thus, the distribution of antibacterial strains varies from area to area. The actual reason for the suppression of antibiosis in Tokyo Bay remains unknown. However, it could be that in shallow areas such as Tokyo Bay, organic nutrients derived from metabolic processes or death and decay of a massive phytoplankton population and constant nutritional inputs from external sources assuage the necessity for bacterial populations to produce antibiotic compounds to survive competition.

Among the various marine animals tested so far, it has been shown that sponges are a good source for antimicrobial compounds (4). Of the 16 isolates from Caribbean sponges, 7 inhibited growth of the assay bacteria (9). However, in the present study, only two strains having antibacterial activity were isolated from sponges.

The isolates showed varied spectra of activity, inhibiting closely related and taxonomically distant microorganisms. This may be due to the fact that the same strain might

TABLE 4. Spectra of activity of the 37 producers against type cultures

Producer	Activity against type culture[a]												
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}
1	+	+	+	+	+	+	+	+	+	+	+	+	+
2	−	+	+	+	+	+	+	−	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	−	+	−	−	−	−	−	−	+	−	+
5	−	−	−	−	−	−	−	−	−	−	+	−	−
6	−	+	−	−	+	+	+	+	+	−	+	+	+
7	−	+	−	−	−	−	−	−	−	+	+	−	+
8	−	−	−	−	−	−	−	−	−	−	+	−	−
9	−	+	−	−	−	−	−	−	+	+	+	−	+
10	+	+	−	+	−	−	−	+	+	+	+	−	+
11	+	+	−	+	−	−	−	+	−	−	+	−	−
12	−	−	−	−	−	−	−	−	−	−	−	−	+
13	+	+	+	+	−	−	+	+	−	+	+	−	+
14	−	+	+	+	+	+	+	−	−	+	+	+	−
15	−	+	+	−	−	−	−	−	−	+	+	−	−
16	−	−	−	−	−	−	−	−	−	−	+	−	−
17	−	−	−	−	−	−	−	−	−	−	+	−	−
18	−	−	−	−	−	−	−	−	−	−	+	−	−
19	−	−	−	−	−	−	−	−	−	−	+	−	+
20	−	+	−	−	−	−	−	−	−	−	+	−	−
21	+	−	+	−	−	−	−	+	−	−	+	−	+
22	+	+	+	−	+	−	−	+	−	−	+	−	+
23	+	+	+	−	−	−	−	+	−	−	+	−	+
24	−	−	−	−	−	−	−	−	−	−	+	−	−
25	−	−	−	−	−	+	−	−	−	−	+	+	+
26	−	+	−	+	+	+	+	−	+	+	+	+	−
27	−	+	−	+	+	+	+	−	+	+	+	+	+
28	−	+	−	−	+	+	+	−	−	−	+	+	+
29	−	−	−	−	−	−	−	+	−	−	+	+	+
30	−	+	−	−	−	−	−	−	+	+	−	−	+
31	−	+	+	+	+	+	−	−	+	+	−	+	+
32	−	−	−	−	−	−	−	−	−	−	−	−	+
33	−	+	−	+	+	+	+	−	−	+	−	+	+
34	−	−	+	+	−	−	−	−	+	−	+	−	−
35	−	−	−	−	−	−	−	−	−	−	+	−	−
36	+	+	−	+	+	+	+	−	−	+	−	+	+
37	−	+	−	−	−	−	+	−	−	−	−	+	+

[a] T_1, *Lucibacterium harveyi* NCMB 42; T_2, *Arthrobacter crystallopoietes* ATCC 15481; T_3, *Pseudomonas putida* ATCC 12633; T_4, *Vibrio anguillarum* ATCC 18264; T_5, *Mycobacterium* sp.; T_6, *Bacillus cereus* var. *mycoides*; T_7, *Sarcina lutea*; T_8, *Escherichia coli*; T_9, *Pseudomonas fluorescens*; T_{10}, *Aeromonas hydrophila*; T_{11}, *Staphylococcus aureus* P 209; T_{12}, *Micrococcus luteus* TAM 1056; T_{13}, *Vibrio parahaemolyticus* ATCC 17802. +, Activity; −, no activity.

TABLE 5. Spectrum of antibacterial activity of producers against producers

Producer	Water													Phytop				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Water																		
1	+																	
2		+			+													
3																		
4	+	+	+							+				+				
5														+				
6																+		
7	+	+	+		+										+			
8	+	+	+		+									+				
9															+			
10			+		+										+			
11		+												+	+			
12	+	+	+								+							
13		+			+													
Phytoplankton																		
14	+				+													
15	+		+															
16																		
17																		
18																		
19																		
20	+	+	+							+	+		+	+				
21																		
22																		
23																		
Animal/zooplankton																		
24																		
25		+	+											+				
26																		
27														+				
28	+		+		+							+						
29		+		+	+							+		+	+			
30																		
31																		
32		+												+				
33																		
34																		
35																		
36																		
Sediment																		
37	+									+								

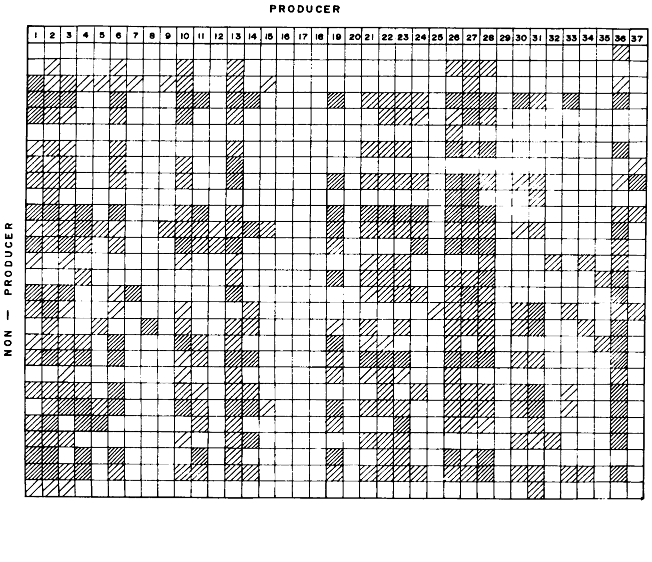

FIG. 2. Inhibition of marine nonproducers by producers. Inhibition ranged from none (□), poor (▨), moderate (▨), to strong (▨).

produce different antibacterial compounds, depending on the culture medium (15). However, the production of antimicrobial compounds might be advantageous in competition for food and space, depending on the area and source of initial isolation. Gauthier and Flateau (8) observed that acidic polysaccharide antibiotics produced by marine violet bacteria played an advantageous role in the adhesion to the substrate (5). Autoinhibition was exhibited by three strains. This phenomenon had been observed in marine bacteria (1, 13). Autoinhibition could act as a sort of controlling factor in maintaining species diversity by perhaps allowing population to partially limit itself and coexist with competitors (6).

Among the antibacterial substance-producing isolates obtained, *Pseudomonas* isolates were the dominant strain. Rosenfeld and ZoBell (17) reported that the majority of species exhibiting inhibitory capabilities belonged to the

TABLE 5—*Continued*

lankton					Animal/zooplankton													Sediment
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
									+									
							+	+		+	+	+					+	
														+				
					+													
																	+	
							+	+			+	+		+				
																	+	
													+					
							+	+		+		+					+	
											+	+		+				
												+						
					+				+								+	
																	+	
							+										+	
							+											
					+		+	+	+		+	+		+			+	
																	+	
																	+	
							+	+			+	+		+			+	
					+			+										
					+		+					+		+				
							+		+									
												+		+				
								+										
							+	+				+		+	+		+	

gram-positive bacteria. Nonetheless, later studies showed that gram-negative producers also occur (7). Okami and Okazaki (16) studied these bacteria from different locations of the Sea of Japan and found that 35% of *Pseudomonas* showed antimicrobial activity. A high degree of antibacterial activity (81.0%) was obtained from *Pseudomonas*/*Alteromonas* isolates in the present study, suggesting that this genus would be interesting in future studies of marine antibacterial compound-producing strains.

TABLE 6. Classification of antibacterial substance-producing bacteria

Source	No. of antibacterial strains in genus[a]		
	Pseudomonas/*Alteromonas*	*Vibrio*	*Flavobacterium*
Water	12		1
Sediment	1		
Phytoplankton	5	5	
Zooplankton	10	1	
Sponges	2		

[a] In all, 81% were *Pseudomonas*/*Alteromonas* isolates, 16.3% were *Vibrio* isolates, and 2.7% were *Flavobacterium* isolates.

Pigments have been associated with antibacterial activity, as is the case for cyanobacteria (13). A high percentage of pigmented bacteria with antibacterial activity was reported by Shiba and Taga (19). However, in the present study, only 30% of the producers were pigmented and these did not show much variation in their spectra of activity compared with that of the nonpigmented. It was seen that gram-negative nonpigmented forms had antibacterial properties, with *Pseudomonas*/*Alteromonas* forming the dominant genus.

The distribution of these bacteria varied with location and source. Their marked absence or presence in low numbers at Tokyo Bay suggest that high nutrient levels may not necessitate or inhibit synthesis of antibacterial compounds in bacteria. It is also possible that these microorganisms are not competing for the same nutrients or other resources. Among the three bays selected, Tokyo Bay was highly eutrophicated, with frequent outbreaks of red tide (10), compared with the less fertile Sagami and Suruga Bays. Further studies on the comparison of antagonistic properties in both copiotrophs and oligotrophs from their respective realms would help confirm the above hypothesis linking elaboration of antagonistic properties to oligotrophy.

ACKNOWLEDGMENTS

S.N. received a scholarship from MONBUSHO (Japanese Government).

We thank R. R. Colwell, University of Maryland, for her valuable suggestions and constant encouragement. Thanks are also due to the captain and crew of R.V. *Tanseimaru* for helping with the sampling.

LITERATURE CITED

1. **Andersen, R. J., M. S. Wolfe, and N. J. Faulkner.** 1974. Autotoxic antibiotic production by a marine *Chromobacterium*. Mar. Biol. **27**:281–285.
2. **Baam, B. R., N. M. Gandhi, and Y. M. Freitas.** 1966. Antibiotic activity of marine microorganisms. Helgol. Wiss. Meeresunters. **13**:181–185.
3. **Buck, J. D., and S. P. Meyers.** 1965. Antiyeast activity in marine environment. 1. Ecological consideration. Limnol. Oceanogr. **10**:385–391.
4. **Burkholder, P. R.** 1973. The ecology of marine antibiosis and coral reef. *In* O. A. Edean (ed.), Biology and geology of coral reefs, vol. 2. Academic Press, Inc., New York.
5. **Corpe, W. A.** 1973. Properties of polyanionic carbohydrates isolated from marine bacteria and the marine environment. Symp. Environ. Biogeochem. **22**:11–12.
6. **Freitas, M. D., and A. G. Fredickson.** 1978. Inhibition as a factor in the maintenance of the diversity of microbial ecosystems. J. Gen. Microbiol. **106**:307–320.
7. **Gauthier, M. J.** 1969. Substances antibactériennes produites par les bactéries marines. I. Étude systematique de l'activité antagoniste de souches marines vis-à-vis de genres telluriques aérobies. Revue Int. Oceanogr. Med. 1–16, 41–59.
8. **Gauthier, M. J., and G. N. Flateau.** 1976. Antibacterial activity of marine violet-pigmented *Alteromonas* with special reference to the production of brominated compounds. Can. J. Microbiol. **22**:1612–1619.
9. **Gjessing, H. W., and R. Ruskin.** 1977. Antibiotic activity of bacteria isolated from some Caribbean sponges, p. 12–14. Proceedings of the 12th meeting of the Association of Island Marine Laboratories of the Caribbean Curaivo (Netherlands Antilles) Sept. 1976. Association of the Institute of Marine Laboratories of the Caribbean.
10. **Izuhara, S.** 1985. The results of a survey of maximum densities in cell numbers of phytoplankton in coastal waters of Japan. Bull. Plankton Soc. Jpn. **32**:67–72.
11. **Krasilnokova, D. N.** 1961. On antibiotic properties of microorganism isolated from various depths of the world oceans. Microbiol. **30**:545–550.
12. **Lebedeva, M. N., and E. M. Markianovic.** 1971. Antibiotic features of heterotrophic bacteria in southern seas, p. 335–352. Proceedings of the 5th International Colloquium of Medical Oceanography.
13. **Lemos, M. L., A. Toranzo, and J. L. Barja.** 1985. Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microb. Ecol. **11**:149–164.
14. **Okami, Y.** 1977. p. 245–255. *In* S. Motoda (ed.), Proceedings of the 5th Japan-Soviet Joint Symposium on Aquaculture, Tokyo (Japan) Sept. 1976. Tokai University, Tokyo.
15. **Okami, Y.** 1984. Marine microorganisms as a source of bioactive substances, p. 651–655. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
16. **Okami, Y., and T. Okazaki.** 1972. Studies on marine microorganisms. I. Isolation from the Japan Sea. J. Antibiot. **25**:456–460.
17. **Rosenfeld, W. D., and C. E. ZoBell.** 1947. Antibiotic production by marine organisms. J. Bacteriol. **54**:393–398.
18. **Saz, A. V., S. Watson, S. R. Brown, and D. L. Lowery.** 1963. Antimicrobial activity of marine waters. 1. Macromolecular nature of antistaphylococcal factor. Limnol. Oceanogr. **8**:63–67.
19. **Shiba, T., and N. Taga.** 1980. Heterotrophic bacteria attached to seaweeds. J. Exp. Mar. Biol. Ecol. **47**:251–258.
20. **Simidu, U.** 1974. p. 45–55. *In* N. Taga (ed.), Marine microbiology. Tokyo University Press, Tokyo. (In Japanese)
21. **Simidu, U.** 1985. p. 228–233. *In* H. Kadota and N. Taga (ed.), Methods in marine microbiology.