STUDIES ON MARINE MICROORGANISMS. I

ISOLATION FROM THE JAPAN SEA

YOSHIRO OKAMI and TAKAO OKAZAKI

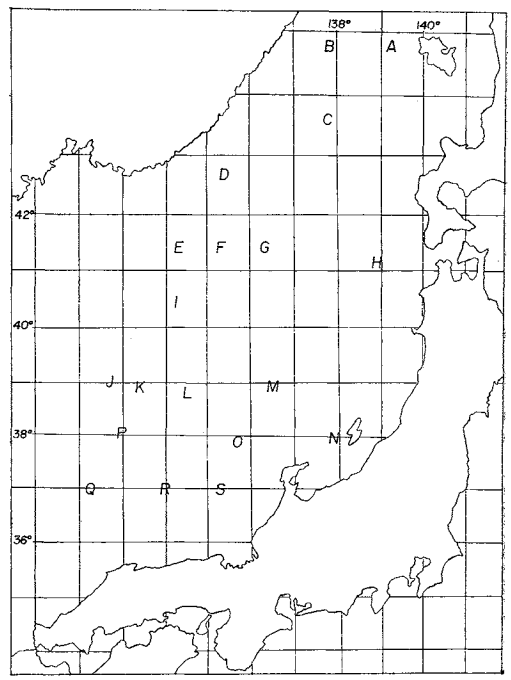
Institute of Microbial Chemistry, Shinagawa-ku, Tokyo, Japan

(Received for publication May 8, 1972)

In this study, 58 samples of water and mud were collected at 19 different locations in the Japan Sea, and from these 142 strains of microorganisms were isolated. In all 126 isolates were bacteria and 16 isolates were yeast and fungi. No actinomycetes were isolated. The isolates were placed into 10 groups in accordance with SHEWAN's scheme. A total of 35 strains (28.5%) of 123 isolates were dependent to sea water, and 25 of these strains (20.3%) showed antimicrobial activities.

Few examples of antibiotic activities from marine microorganisms have been reported[4,5,6], while various kinds of antibiotics have been obtained mainly from different terrestrial microorganisms. Since environmental conditions of the sea are extremely different from terrestrial conditions, it is supposed that microorganisms in the sea have different characteristics from known terrestrial microorganisms and hence, might produce different types of antibiotics from those we know. Microorganisms were isolated from samples collected in the Japan Sea and placed by their characteristics into 10 groups. Their antibiotic activity was examined under various conditions.

Fig. 1. Locations in the Japan Sea for the collection of sea water and mud.



Materials and Methods

In all, 58 samples were collected with the help of the Science and Technology Agency and the Maritime Safety Agency of Japan. Sea water and mud were collected at 19 different locations (Table 1, Fig. 1) in the Japan Sea during the cruise of coast guard vessel "Takuyo-maru" in August, 1969.

Sampling Methods: Water samples were collected with the aid of NANSEN's sampler and mud samples with a core sampler. These samples were aseptically stored in 250 ml polyethylene bottles and kept in a cold box (5°C) during the cruise.

Methods of Isolating Microorganisms: A 100-ml aliquot of each water sample was centrifuged at 1,000 rpm for 10 minutes

and aseptically filtered with a Millipore membrane filter (pore size 0.3 μ, PH type). Concentrated microorganisms on the filter were suspended in 2.5 ml of the filtered sea water. A 0.5-ml aliquot of the suspension was spread over four kinds of agar media of different composition (Fig. 2). These media were cultured for one to two weeks at 22°C, and the colonies which appeared were selected and transplanted to an agar slant of the same composition as above. Sea mud samples were spread directly over the agar media.

Medium	Components (%)	
K	Glucose	1.0
	Asparagine	0.05
	K_2HPO_4	0.05
	Agar	1.7
	pH 7.4	
MYS	Maltose	1.0
	Yeast extract	0.4
	Agar	1.7
	pH 7.2	
ZD	Bacto-peptone	0.5
	Ferric phosphate	0.01
	Bacto-yeast extract	0.1
	Agar	1.7
	pH 7.6	
Z	Same as ZD (artificially mixed sea water* instead of distilled water in ZD)	
	* Jamarine Co., Ltd., Osaka	

Antimicrobial Activity: Isolates were inoculated into the following three media and shaken at 22°C for 4 days. The broths were examined for antimicrobial activity by the cylinder plate method against following test microorganisms, *Staphylococcus aureus* 193, *Escherichia coli* NIHJ, *Mycobacterium* 607, and *Candida albicans* and *Phytophthora capsici*.

Medium	Components (%)	
V	Glucose	0.5
	Peptone	1.0
	Yeast extract	0.5
	K_2HPO_4	0.05
	$MgSO_4$	0.05
	NaCl	2.0
	pH 7.8	

Table 1. Location in the Japan Sea for the collection of sea water and mud with reference to group of the isolated microorganisms.

Station	Date	North latitude	East longitude	Depth (m)	Group**
A	44. 7. 1	44°45′	139°20′	Surface	F_1
				400	Yeast, Fungi
				1, 000	Fungi
				1, 520*	B, D, Fungi
B	7. 2	44°45′	137°52′	Surface	—
				400	—
				1, 000	—
C	7. 3	43°27′	137°44′	Surface	—
				400	F_1
				1, 000	F_2
D	7. 5	42°29′	135°23′	Surface	F_1
				400	B
				1, 000	F_4
E	7. 6	41°16′	134°16′	Surface	F_1, F_2
				400	Fungi
				1, 000	A, F_1
F	7. 6	41°16′	135°11′	Surface	B, Fungi
				400	F_1, F_2
				1, 000	B, Fungi
G	7. 7	41°14′	136°18′	3, 300	—
				3, 720*	B
H	7. 8	41°01′	138°55′	Surface	F_3, Fungi
				400	D
				1, 000	—
I	7. 14	40°16′	134°13′	Surface	A, B, D, F_4
				400	A, B
				1, 000	F_1, F_2
J	7. 15	38°53′	132°45′	Surface	Fungi
				400	F_4
				1, 000	A, D
K	7. 15	38°44′	133°22′	Surface	A
				400	Fungi
				1, 000	F_3
L	7. 16	38°37′	134′30′	2, 950*	C, D, F_3
M	7. 16	38°51′	136°33′	Surface	—
				400	D
				1, 000	A, F_4
				2, 630*	B, F_1, F_2
N	7. 17	37°45′	137°52′	Surface	D, Fungi, Yeast
				400	A, Yeast
				1, 000	F_4
				1, 640	A, B, C, D, F_4
O	7. 19	37°43′	135°42′	Surface	F_1, F_2, Fungi
				400	—
				1, 000	A
				2, 830*	A, Fungi
P	7, 19	37°54′	133°02′	Surface	A, B
				400	F_3, Fungi
Q	7. 21	36°49′	132°16′	Surface	A, B, D, F_2
				400	A, B, F_3
				1, 000	A, B, C, F_1
				1, 540*	C, F_1, F_4
R	7. 22	36°53′	134°02′	Surface	Yeast
				400	F_3
				1, 000	F_4
				1. 720	A, C, F_2
S	7. 22	36°53′	135°16′	Surface	B, F_4
				400	F_1
				1, 000	B

* sea mud. ** group designated in Table 2.

Fig. 2. Isolation of marine microorganisms from sea water.

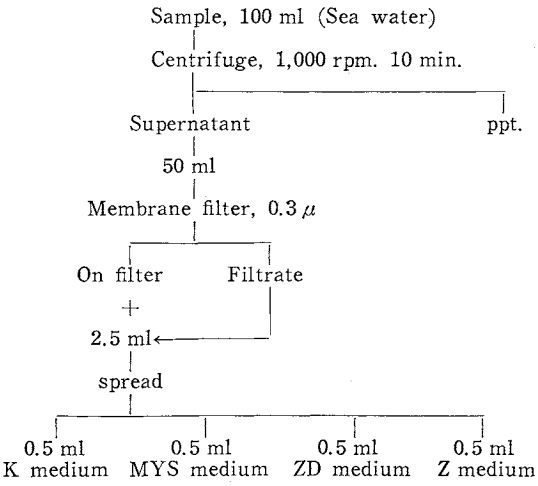


Table 2. Classification of isolates from the Japan Sea

Group	Main genera	Number of isolates
A	*Paracolobacterium Escherichia*	22
B	*Achromobacter Alcaligenes*	18
C	*Flavobacterium, Cytophaga*	14
D	*Vibrio*	12
E	*Aeromonas*	0
F_1	*Pseudomonas* type 1	15
F_2	*Pseudomonas* type 2	6
F_3	*Pseudomonas* type 3	6
F_4	*Pseudomonas* tyde 4	14
G	Strains not grouped in SHEWAN's scheme	19
Yeast		5
Fungi		11
Total		142

Table 3. Growth dependence to sea water

Group	Tested	Dependent*
A	22	4
B	18	5
C	13	3
D	11	2
E	0	0
F_1	15	4
F_2	6	2
F_3	6	4
F_4	14	7
G	13	4
Yeast	5	0
Fungi	0	0
Total	123	35

* No growth in nutrient broth (Distilled water is used instead of sea water).

Table 4. Antimicrobial activity of marine microorganisms

Group	Tested	Active*	Active against microorganisms**			
			Staph.	*E. coli*	*Mycob.*	Fungi
A	22	4				4
B	18	3			1	2
C	13	1				1
D	11	2		1		1
F_1	15	5		1	1	3
F_2	6	2		1		1
F_3	6	2				2
F_4	14	5		1	3	4
G	13	0				
Yeast	5	1		1		1
Total	123	25	0	5	5	19

* Active to either bacteria or fungi tested.
** *Staph.* : *Staphylococcus aureus* 193.
E. coli : *Escherichia coli* NIHJ.
Mycob. : *Mycobacterium* 607.
Fungi : *Candida albicans* and *Phytophthora capsici*.

X		
	Glucose	0.5
	Soy bean meal	1.5
	K_2HPO_4	0.05
	$MgSO_4$	0.05
	NaCl	2.0
	pH 7.8	

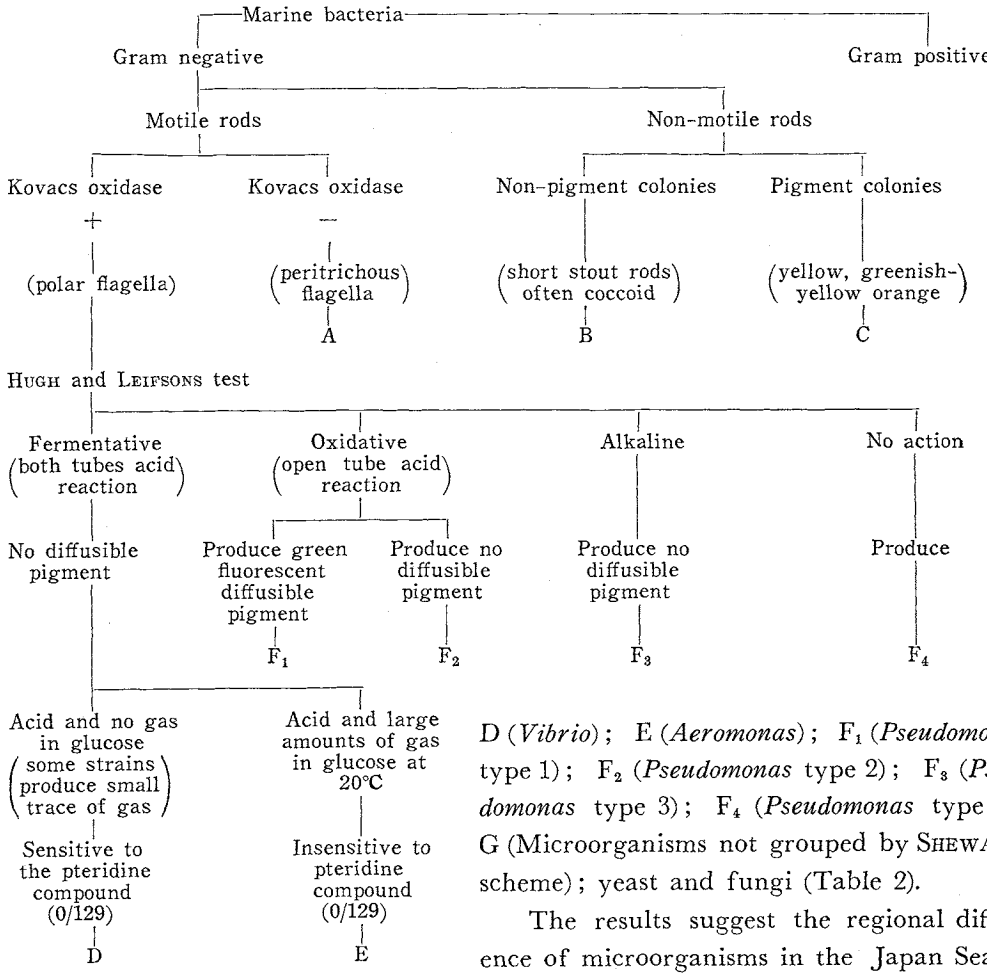
Z		
	Bacto-peptone	0.5
	Bacto-yeast exract	0.01
	Ferric phosphate	0.1
	Artificially mixed sea water 750 ml and dist. water 250 ml	
	pH 7.8	

Results and Discussion

Grouping of Isolated Marine Microorganisms

On the basis of morphological and physiological characteristics, 142 isolated strains were placed into 10 groups namely, from A to G according to SHEWAN's scheme[1] (Fig. 3). Each of the groups contains the following genera; A (*Paracolobacterium, Escherichia*); B (*Achromobacter, Alcaligenes*); C (*Flavobacterium, Cytophaga*);

Fig. 3. Classification scheme of marine bacteria



D (*Vibrio*); E (*Aeromonas*); F_1 (*Pseudomonas* type 1); F_2 (*Pseudomonas* type 2); F_3 (*Pseudomonas* type 3); F_4 (*Pseudomonas* type 4); G (Microorganisms not grouped by SHEWAN's scheme); yeast and fungi (Table 2).

The results suggest the regional difference of microorganisms in the Japan Sea as shown in Table 1. Among the isolates, bacteria, especially Gram-negative bacteria, were seen more often than other microorganisms such as fungi and yeast. No actinomycetes were isolated from the Japan Sea in this experiment, while many actinomycetes have been isolated by other investigators from the North Atlantic Ocean and the Pacific Ocean[2,3,4]. Failure to isolate actinomycetes would be partly dependent on the period preserving samples at 5°C more than one year and partly on the scarcity of actinomycetes in the deep sea.

Growth Dependence on Sea Water

In all, 123 strains of isolated marine microorganisms were inoculated onto two media, Bacto-nutrient broth (Bacto-beef extract 0.3%, Bacto-peptone 0.5%, pH 6.8) and sea water Bacto-nutrient broth (prepared artificially with sea water instead of distilled water as above) and cultured for 1 week at 22°C. As shown in Table 3, 35 strains (28.5%) were dependent on sea water.

Antimicrobial Activity of Marine Microorganisms

There have been few reports on the antimicrobial activity of marine microorganisms[4,5,6]. Here, 123 strains of marine microorganisms were examined for possible

antimicrobial activity by using three kinds of medium. The results are shown in Table 4. It is of interest to note that these marine microorganisms exhibit poor activity against Gram-positive bacteria in comparison with the terrestrial microorganisms ever isolated. The fact that more than 20 percent of marine microorganisms tested showed antimicrobial activities, suggests that they would be worthy to study as sources for biologically active principles.

Acknowledgements

The authors wish to thank deeply the Science and Technology Agency and the Maritime Safety Agency of Japan for their help in collecting samples. They also wish to thank Dr. H. UMEZAWA for his valuable advice and encouragement, and Miss H. NAKAMURA and Miss H. SHIMAZU for their technical assistance.

References

1) SHEWAN, J. M. : The differentiation of certain genera of Gram-negative bacteria frequently encountered in marine environment. In C. H. OPPENHEIMER (ed.), Marine microbiology. Charles C. Thomas, Publisher, Springfield. pp. 499~521, 1963
2) WEYLAND, H. : Actinomycetes in North Sea and Atlantic Ocean sediments. Nature 223 : 858, 1969
3) WEYLAND, H. : Studies on Actinomycetes of the sea benthos. Presented at the Ocean World-Joint Oceanographic Assembly, Tokyo, 1970
4) GREIN, A. & S. P. MEYERS : Growth characteristic and antibiotic production of Actinomycetes isolated from littoral sediments. J. Bact. 76 : 457~463, 1958
5) ROSENFELD, W. D. & C. E. ZOBELL : Antibiotic production by marine microorganisms. J. Bact. 54 : 393~398, 1947
6) BURKHOLDER, R. & G. M. SHARMA : Antimicrobial agents from the sea. Lloydia 32 : 466~483, 1969