THE MEMBRANE FILTER IN MARINE MICROBIOLOGY[1]

CARL H. OPPENHEIMER

Scripps Institution of Oceanography, University of California, La Jolla, California

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The advent of the membrane filter has opened a new path for the study of bacteria. The apparatus and procedures have been described by Clark *et al.* (1951) and Goetz and Tsuneishi (1951).

The membrane filter used in these experiments employs a filter membrane consisting of a mixture of cellulose esters which can be carefully controlled to provide different effective pore sizes (Goetz, 1951). The effective pore size used in the following experiments is referred to as "Z" value which describes the specific flow resistance. Z is determined by the time required for one ml of distilled water to pass through one cm of filter area at a pressure of 70 cm of Hg. A filter of Z = 0.8 to 1.0 seconds has an effective pore size of approximately 0.4 microns. The filter type Z = 1.5 to 2.0 seconds (table 1) was able to retain marine bacteria while a filter type Z = 0.8 to 1.0 seconds capable of complete retention of fresh water-born microorganisms proved to allow certain marine bacteria to pass. The filter membranes were sterilized with ethylene oxide vapor for four hours in a closed system. The abundance and kinds of bacteria retained by the filter may be determined either by cultural procedures or by their direct microscopic observation. The latter can best be accomplished with a phase contrast microscope after rendering the filter membrane transparent with an inert, neutral oil having a refractive index of 1.49.

With the use of appropriate nutrient media and conditions of incubation, either aerobes or anaerobes can be cultivated on the surface of the filter membranes. The nutrient sea water media for the membrane filter technique were concentrated by using from two to four times the normal solid ingredients per volume of water. After quantitatively removing the bacteria from a water sample by its passage through a filter membrane, the effluent side of the latter was placed on a sterile absorbent pad soaked with suitable nutrient medium. The bacteria on the surface of the membrane obtain the soluble nutrients from the pad below by capillary attraction and diffusion. After a few hours' incubation in a moist chamber at 25 C, microcolonies resulting from multiplying bacteria can be counted with a low power dissecting microscope. The colonies appear more rapidly on the filter membrane than on standard agar or gelatin plates. If enumeration must be delayed, the colonies on the membranes may be preserved by formaldehyde vapors and kept for counting later.

For the experiments on aerobic bacteria, nutrient sea water medium as described by ZoBell (1946) was used in concentrated form. The oxidation-reduction indicator, 2,3,5-triphenyl-tetrazolium chloride as used by Goetz and Tsu-

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TABLE 1

The filtering efficiency of the Z = 0.8 to 1.0 and Z = 1.5 to 2.0 second filter membranes for marine bacteria naturally present in sea water as determined by bacterial counts with nutrient sea water medium

FILTER MEMBRANE	SAMPLE NO.	VIABLE CELLS/ML IN SEA WATER	VIABLE CELLS/ML IN FILTRATE
Z = 0.8 to 1.0	1	3,700	5
	2	10,000	0
	3	4,500	4
	4	4,300	4
	5	10,000	12
	6	8,500	4
Z = 1.5 to 2.0	1	1,150	0
	2	950	0
	3	914	0
	4	991	0

TABLE 2

The effect of 2,3,5-triphenyl-tetrazolium chloride (TPTZ) as an oxidation-reduction indicator on the growth of marine bacteria as expressed by viable cell counts with the MF and plating methods

SAMPLE NO.	TPTZ PRESENT MF METHOD CELLS/ML	TPTZ ABSENT PLATING METHOD CELLS/ML
1	1	230
2	2	280
3	140	670
4	100	914
5	112	991
6	180	950

TABLE 3

The minimum number of viable bacteria in different sea water samples when incubated at 25 C as determined by the MF method and the standard plating or dilution methods

MEDIA	SAMPLE NO.	MF METHOD	PLATE OR DILUTION METHOD
Sea water broth (aerobic)	1	7,800	5,500
	2	2,700	1,430
	3	3,400	970
	4	110	130
	5	16	2
	6	2	2
	7	3	2
SO_4-reducing (anaerobic)	1	150	62
	2	370	0

neishi (1951), was incorporated in the sea water medium in earlier experiments. As table 2 indicates, 2,3,5-triphenyl-tetrazolium chloride inhibited approximately 90 per cent of the marine bacteria which would normally grow in its absence. When 2,3,5-triphenyl-tetrazolium chloride was omitted in later experi-

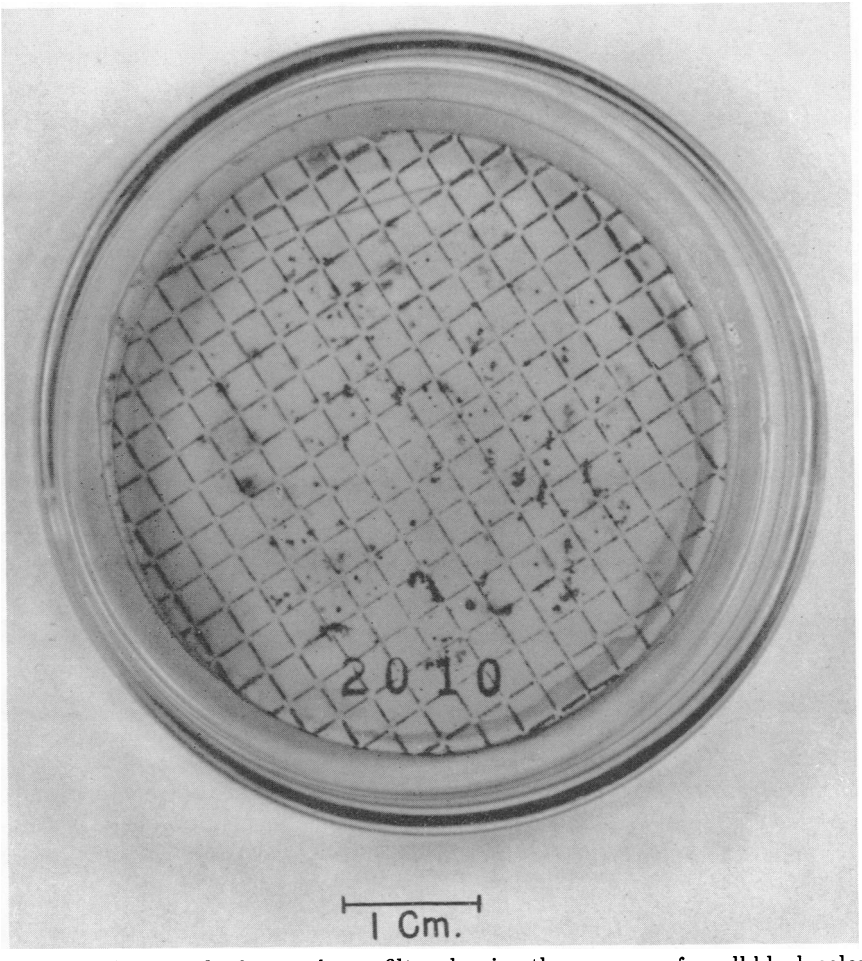

Figure 1. Photograph of a membrane filter showing the presence of small black colonies of sulfate reducing bacteria as filtered from 10 ml of raw sea water after 7 days' incubation at 25 C under anaerobic conditions.

ments (table 3), it was found that approximately 30 per cent more bacterial colonies per unit of water developed on membranes treated with nutrients than on agar plates containing comparable nutrients. The higher colony counts obtained on the membranes are believed to be due in part to the fact that some marine bacteria are injured by exposure to the plating temperatures of agar (ZoBell and Conn, 1940).

Anaerobes may be cultivated on the bacteria-laden membranes when the latter are placed on pads soaked with appropriate nutrient medium as described by Sisler and ZoBell (1950). After the membranes were incubated for seven days in a moist chamber at 25 C from which oxygen was excluded, preferably by the chromium-sulfuric acid method, even fastidious sulfate reducing bacteria developed colonies. These could be distinguished from colonies of other anaerobes by the black zone formed by the reaction of hydrogen sulfide with iron in the medium (figure 1). The cloudy black zone which is associated with the growth of sulfate-reducers in agar media and which tends to mask the original colony site was absent. Therefore the colonies on the filter membrane were easily detected. In these experiments (table 3), from two to three times as many marine sulfate reducers were detected as could be discerned by the minimum dilution method. The colonies of sulfate reducers appeared under the microscope to be small and raised, having a metallic sheen with concentric black rings. This method may prove to be valuable for obtaining pure cultures of the sulfate reducing bacteria, whereby single colonies may be picked from the surface of the membrane.

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SUMMARY

This new technique affords some manifest advantages. It is an excellent tool for sampling marine psychrophilic bacteria which constitute a major portion of the bacteria in the sea. The results would indicate that higher numbers of bacteria capable of multiplying on sea water broth may be present in sea water than may be determined by standard methods. In addition, the small amount of necessary equipment, the exclusion of solidifying agents, and ease of manipulation make the equipment highly desirable for use at sea.

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