動植物プランクトンが海洋細菌の増殖に与える効果について

誌名	日本水産學會誌
ISSN	00215392
著者	木暮, 一啓 清水, 潮 多賀, 信夫
巻/号	46巻3号
掲載ページ	p. 323-326
発行年月	1980年3月

Bulletin of the Japanese Society of Scientific Fisheries 46(3), 323–326 (1980)

Effect of Phyto- and Zooplankton on the Growth of Marine Bacteria in Filtered Seawater

Kazuhiro KOGURE*, Ushio SIMIDU, and Nobuo TAGA

(Received October 4, 1979)

Living phyto- or zooplankton was isolated aseptically from natural seawater and incubated in seawater, enriched with small amounts of nitrogen and phosphorus sources under controlled conditions. The effect of the added plankton on the growth of heterotrophic bacteria and *Vibro* sp. in particular, were assessed. The overall results showed that generally phytoplankton specifically suppressed the growth of *Vibrio* sp., while zooplankton enhanced it. These effects were clear in samples incubated for 12 or 24 hours. The inhibitory effect of the phytoplankton became less remarkable after a longer period of incubation.

In a marine environment there are various specific interactions between phytoplankton and bacteria.[2,4,5,7–9] Based on field observations and experiments with natural seawater, several investigators have suggested that phytoplankton populations specifically inhibit the growth of *Vibrio* in natural seawaters.[9,11] These studies, however, did not give any direct evidence for the suppression of *Vibrio* growth by phytoplankton populations. In natural seawaters, there are not only phytoplankton-bacterial interactions, but also bacteria-bacterial or zooplankton-bacterial interactions, which make it difficult to interpret the data.

We reported that the marine diatom, *Skeletonema costatum* suppressed the growth of *Vibrio* and *Pseudomonas* in mixed cultures using an artificial culture medium.[6] This result, however, cannot be directly applied to *in situ* conditions. The object of the present study is to clarify the effect of naturally occurring plankton on the growth of heterotrophic bacteria, especially *Vibrio* sp. in natural seawater that is free from other organisms. Phytoplankton was isolated aseptically by repeated washings with capillary tubes. Cells thus obtained were inoculated into natural seawater samples and incubated for several days under controlled conditions. The growth of heterotrophic bacteria and *Vibrio* sp. in the culture media were followed. Likewise, experiments to study the effect of zooplankton on the bacterial growth were carried out, and the results were compared and discussed.

Materials and Methods

Sample seawater and plankton were collected at 35°09′N, 139°17′E in Sagami Bay, Japan, on Dec. 13, 1978 during the KH–78–5 cruise aboard R/V Hakuho-maru (Ocean Research Institute, University of Tokyo). Sterilized glass bottles were used for the sampling of surface seawater. After filtration through Nucleopore filter (pore size 5 μm) to remove most of the plankton and large particles, the seawater was enriched with KH_2PO_4 (4.5 mg/*l*) and KNO_3 (7.2 mg/*l*). Phyto- and zooplankton were obtained with an alcohol-washed XX–13 plankton net (Regosha & Co., Ltd.). Several vertical tows from about 2 m depth to the surface provided sufficient plankton samples. Phytoplankton cells or chains were picked up immediately on board using sterilized capillary tubes under a microscope.[3] Four or five continuous washings with sterile seawater enabled to obtain only phytoplankton cells without any other plankton or particles. Since the zooplankton moved rapidly, it was difficult to pick up one individual using the above method, so zooplankton was gathered on an alcohol-washed GG–54 net (Rigosha & Co., Ltd.) and rinsed several times with sterile seawater. These processes were performed on board within 3 hours after sampling. About 40 cells of phytoplankton or 30 individuals zooplankton were inoculated into 30 m*l* of filtered seawater in 50 m*l* glass bottles with cotton plugs. Phytoplankton samples were incubated at 20°C under continuous 4,000 lux fluorescent illumination. The zooplankton and control samples were incubated in the dark at 20°C.

To determine the viable bacterial counts in the natural seawater used in the experiment, the appropriate amounts of the duplicate seawaters were

* Ocean Research Institute, University of Tokyo; Nakano, Tokyo 164, Japan (木暮一啓: 東京大学海洋研究所).

filtered through Nuclepore filters (pore size 0.2 μm), which were then incubated on Taga's PPES-II agar media[13] at 20°C for 2 weeks. Viable counts of heterotrophic bacteria in the culture medium were determined by the spread-plate method using the same medium. The number of *Vibrio* sp. was determined at the same time by an anaerobic culture method.[12]

Results and Discussion

The phytoplankton isolated and incubated is listed in Table 1. After 3 days incubation, *Stephanopyxis palmeriana* (Greville) Grunow showed the most prominent growth among them. *Coscinodiscus* sp. had died away during that period. About four-fifths of the zooplankton isolated and incubated was *Paracalanus parvus* and most of the remainder were *Oithona brevicornis*. The zooplankton gradually died during incubation and few of them survived the 3 days.

Table 1. Isolated phytoplankton

Chaetoceros sp.
Coscinodiscus sp.
Ditylum sp.
Nitzschia sp.
Stephanopyxis sp.

Figure 1 shows the growth of heterotrophic bacteria in each culture medium. The presence of zooplankton caused a rapid increase in viable counts, especially during the first 12 hours. Apparently the various organic matter excreted by the zooplankton were quickly utilized as nutrients by bacteria. It is also probable that the dead zooplankton body *per se* served as good substrate for bacteria.

There was little difference between the bacterial growth in the culture with phytoplankton and in the control. Phytoplankton did not seem to induce such a prominent bacterial growth as zooplankton. This may partly due to the relatively low concentration of organic matter in the culture with phytoplankton compared with that bearing zooplankton. It is not clear whether this is due to the antibacterial action of phytoplankton[1,6,9] or low their nutrient value. The bacterial growth in the control might have been sustained by naturally occurring organic substances.

The growth of *Vibrio* sp. enumerated on *Vibrio* medium in the anaerobic culture are shown in Fig. 2. In the culture with zooplankton, rapid

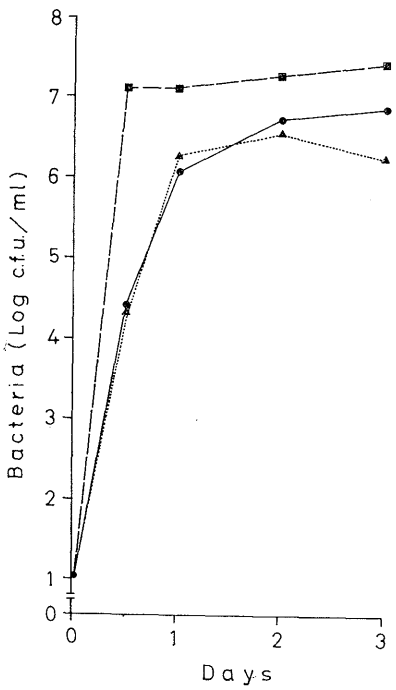

Fig. 1. Growth of heterotrophic bacteria.
Circles: phytoplankton culture; squares: zooplankton culture; triangles: control.

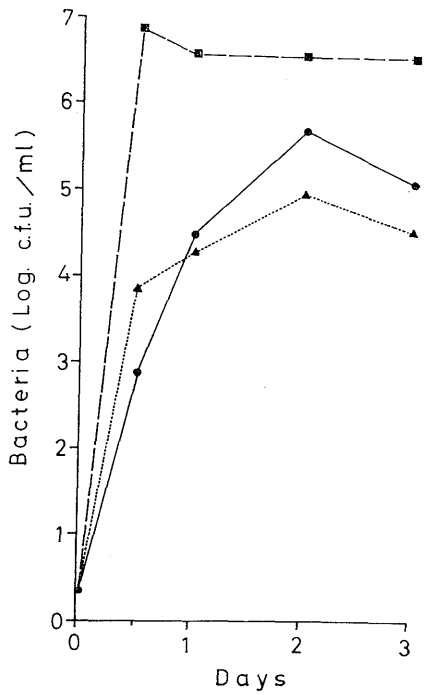

Fig. 2. Growth of *Vibrio* sp.
Symbols are the same as in Fig. 1.

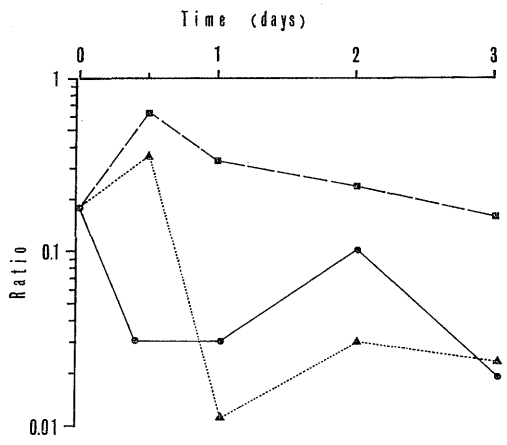

Fig. 3. *Vibrio*-heterotrophic bacteria ratio. Symbols are the same as in Fig. 1.

increases comparable to those of heterotrophic bacteria were observed. On the other hand, initial growth of *Vibrio* sp. in the culture with phytoplankton was low. After 12 hours, the number was less than those in the control by about an order of magnitude. The relative ratios of the number of *Vibrio* sp. to heterotrophic bacteria are shown in Fig. 3. As can be seen in the control, the growth of *Vibrio* is generally characterized by its rapid increase and subsequent sudden decrease in comparison with other bacterial groups. It is clear that phytoplankton specifically suppressed the growth of *Vibrio* at least during the initial stage of the incubation. The inhibitory action was clearly shown in the first day but not thereafter. This result is well in accordance with the observation that the antibiotic action of *S. costatum* on the growth of *Vibrio* or *Pseudomonas* sp. was most prominent after one day's incubation and weakened thereafter.[6] The present results strongly suggest that the naturally occurring phytoplankton cells act more antagonistically to *Vibrio* sp. than the various other bacterial groups in seawater. This is consistent with several field observations.[9,11]

Conversely, zooplankton enhanced the growth of *Vibrio* sp. The ratios in the zooplankton culture were far higher than in the control throughout the incubation period. It has been suggested that the zooplankton have attached or intestine flora dominated by *Vibrio* sp.[10] These *Vibrio* sp. might rapidly outgrow and exceed other bacterial groups in the culture bottle.

Generally, it is very difficult to isolate phytoplankton cells without any contamination of zooplankton or large detrital particles. The size fractionation with several different size nets cannot completely separate the phytoplankton needed. Only by a technique based on repeated washings and isolation, although this required considerable care, did it become possible to isolate particular phytoplankton cells. Immediate treatment of fresh samples and careful control of incubation conditions are necessary. The volume of culture medium should be small for good growth of the inoculated phytoplankton and for obtaining a clear picture of their effect on the bacterial population in the culture bottle.

The result of the present investigation showed that the presence of zooplankton in natural seawater enhances the growth of bacteria, notably *Vibrio* sp. in that seawater. On the other hand, phytoplankton cells specifically suppressed the growth of *Vibrio* sp. in the seawater. For further investigation of the interaction between phytoplankton and bacteria in natural seawater, detailed and elaborate field observations will be necessary. The nutritional value of phyto- or zooplankton bodies for bacteria should be also evaluated. In future, it will be especially important to purify the antibiotic substances excreted by algal cells and to clarify their chemical properties.

Acknowledgements

We are indebted to Mr. S. NISHIDA and Mrs. K. OGAWA, Ocean Research Institute, University of Tokyo, for the identification of plankton samples. We wish to thank the officers and crew of R/V Hakuho-maru, Ocean Research Institute, University of Tokyo, for their kind help during the cruise.

References

1) D. C. B. DUFF, D. L. BRUCE, and N. J. ANTIA: *Can. J. Microbiol.*, **12**, 877–884 (1966).
2) W. BELL, J. M. LANG and R. MITCHELL: *Limnol. Oceanogr.*, **19**, 833–839 (1974).
3) R. W. HOSHAW and J. R. ROSOWSKI: in "Handbook of Phycological Methods" (ed. by J. R. STEIN), Cambridge University Press, 1973, pp. 53–68.
4) E. T. JOLLEY and A. K. JONES: *Br. Phycol. J.*, **12**, 315–328 (1977).
5) E. G. JØRGENSEN and E. S. NIELSEN: *Physiol. Pl.*, **14**, 896–908 (1961).
6) K. KOGURE, U. SIMIDU, and N. TAGA: *J. Exp. Mar. Biol. Ecol.*, **36**, 201–215 (1979).
7) C. E. LUCAS: *Deep-Sea Res.*, **3** (Suppl.), 139–148 (1955).

8) J. McN. SIEBURTH: *Limnol. Oceanogr.*, **4**, 419–424 (1959).
9) J. McN. SIEBURTH: in "Advances in Microbiology of the Sea" (ed. by M. R. DROOP and E. J. F. WOOD), Academic Press, London and New York, 1968, pp. 63–94.
10) U. SIMIDU, K. ASHINO, and E. KANEKO: *Can. J. Microbiol.*, **9**, 1157–1160 (1971).
11) U. SIMIDU, E. KANEKO, and N. TAGA: *Microbial Ecol.*, **3**, 173–191 (1977).
12) U. SIMIDU and K. TSUKAMOTO: *Microbial Ecol.*, (in press)
13) N. TAGA: *Bull. Misaki Marine Biol. Inst. Kyoto Univ.*, **12**, 65–76 (1968).