近畿大農紀要 25： 9～17 (1992)
Mem. Fac. Agr. Kinki Univ. 25： 9～17 (1992)

Size Distribution of Bacterial Cells in Uragami and Tanabe Bays

Mitsuru EGUCHI* and Akira KAWAI*

Synopsis

The size distribution of bacterial cells collected from different three living sites, seawater, particle fractions of seawater (>5 μm), and bottom sediments, in Uragami and Tanabe Bays were investigated with a scanning electron microscope and an image analyzer. Bacterial cells in seawater at both bays tended to be cocci ; however, the cell sizes were different. The mean size of bacterial cells in Uragami Bay was 0.14 μm^2 and that of bacterial cells in Tanabe Bay was 0.17 μm^2. In the particle fractions of seawater, the ratios of the large cells (mean, 0.30 μm^2) and rod shaped cells to total cells were higher and the values of the index of dominance were lower than those of seawater samples. In bottom sediments, the cells were often rods and the cell size was intermediate between those of seawater and the particle fractions of seawater.

Introduction

There are two major living sites for marine bacteria in inland bays. One is seawater, and the other is bottom sediments. Bacterial living sites in seawater are further divided into two kinds ; one is seawater itself, with the bacteria free-living, and the other is particles in seawater, with the bacteria attached. Differences in living sites are associated with taxonomical, morphological, and physiological differences in the bacteria. The ratios of obligate oligotrophs, facultative oligotrophs, and eutrophs to total viable heterotrophic bacteria are different among these three living sites in Uragami and Tanabe Bays[1].

The purpose of this study was to identify differences in the size distributions of bacterial cells depending on differences in the bacterial living sites (seawater, particle fractions of seawater, and bottom sediments) in Uragami and Tanabe Bays.

Materials and Methods

A map of sampling locations is given in Fig. 1. Water depths at Stns. U1, U2, T1, and T2 were 8.0, 8.1, 12.6, and 14.0 m, respectively. Water and bottom sediment samples were collected with a Van Dorn water sampler and a K-K type core sampler (original model ; Kimata et al.[2]), respectively. Samples were immediately preserved by the addition of filtered glutaraldehyde solution to the final concentration of 1.0%, and the samples were stored at 4°C until examined microscopically.

The seawater samples collected at a depth of 0.5 m at Stns. T1 and U1 were fractionated in the laboratory with sterilized nylon nets of 5 μm mesh. Bacterial cells in this fraction were tentative-

* Lab. of Environmental Science for Aquaculture, Dept. of Fisheries, Kinki Univ., Nakamachi, Nara 631, Japan (水産学科水族環境学研究室)

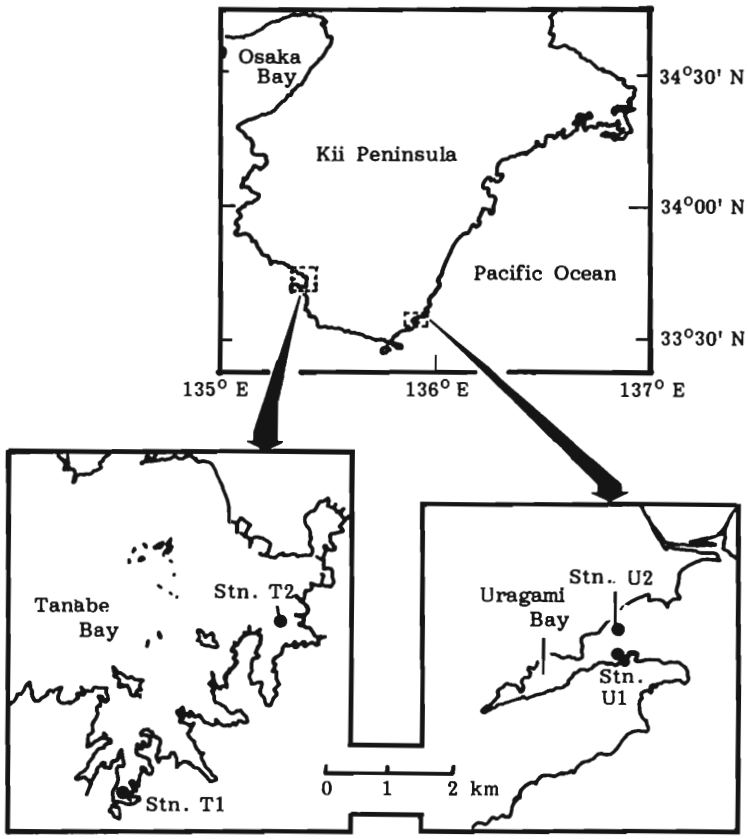

Fig. 1. Sampling locations in Uragami and Tanabe Bays.

ly called "attached bacteria". The ratio of attached bacteria to total bacteria was only a few percents in the two samples investigated here; more than 95% of bacterial cells in the whole seawater samples were "free-living".

For comparison of the cell sizes and shapes, a scanning electron microscope (SEM; JSM-T200, JEOL) was used to take photomicrographs of bacterial cells in each sample (examples in Fig. 2). To prepare samples, they were filtered through a Nucleopore filter with pores of 0.2 μm (filter, 10 mm in diameter); the cells on the filter were transferred to 75, then 50, finally 25% filtered seawater, and dehydrated in 50, 70, 80, 90, twice in 100% ethanol, and then 100% isoamyl acetate, and finally dried at the critical point. The dried filters were sputter-coated with 15-nm Au, and viewed under a SEM at ×5,000-10,000. Photographic prints obtained with the SEM were enlarged, and then the area (μm^2) of each bacterial cell on the prints was measured with an image analyzer (Multi Image Analyzing system, NAC Ltd.).

Based on the histograms of the cell areas and the number of bacterial cells, the index of dominance (c) was calculated from the formula $c=\sum (ni/N)^2$, where ni is the number of bacteria in fraction i, and N is the total number of bacterial cells analyzed.[3] Differences in cell size are more characteristic through use of the cell area rather than the cell length, so the area of cells on the photographic print was used to calculate c[4].

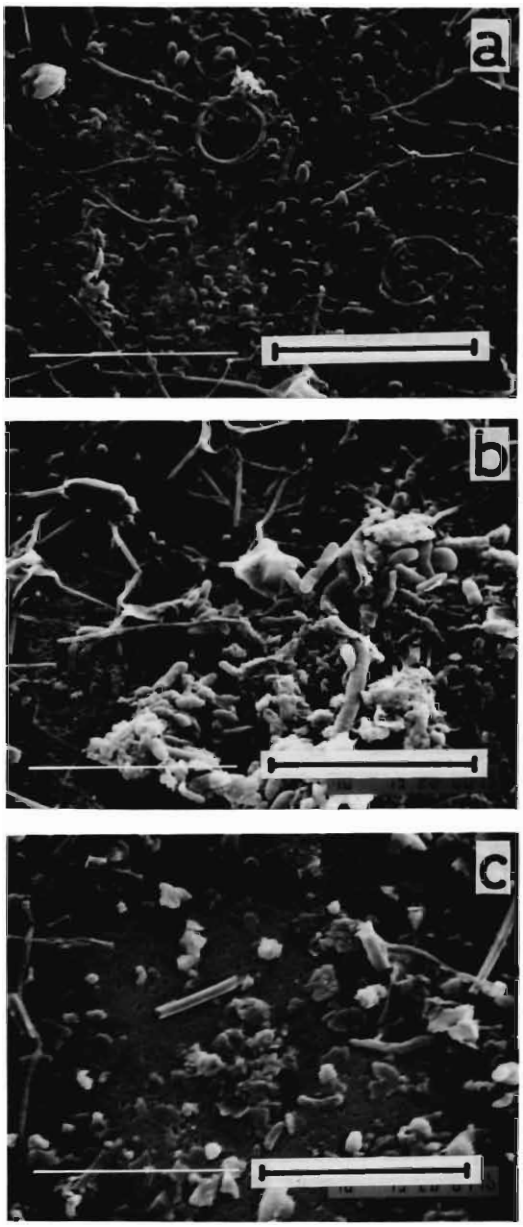

Fig. 2. Typical cell images by SEM at Stn. U1 in Uragami Bay. a, seawater (depth, 0.5 m) ; b, particle fraction of seawater (>5 μm) ; c, bottom sediment. Bars, 10 μm.

Results

Typical SEM photographs of whole seawater, particle fractions of seawater (>5 μm), and bottom sediment are shown in Fig. 2. These photographs were taken of samples collected at Stn. U1 in Uragami Bay. In all samples investigated, more than 80% of bacterial cells in the whole-seawater samples were cocci or short rods but rods were most common in particle fractions

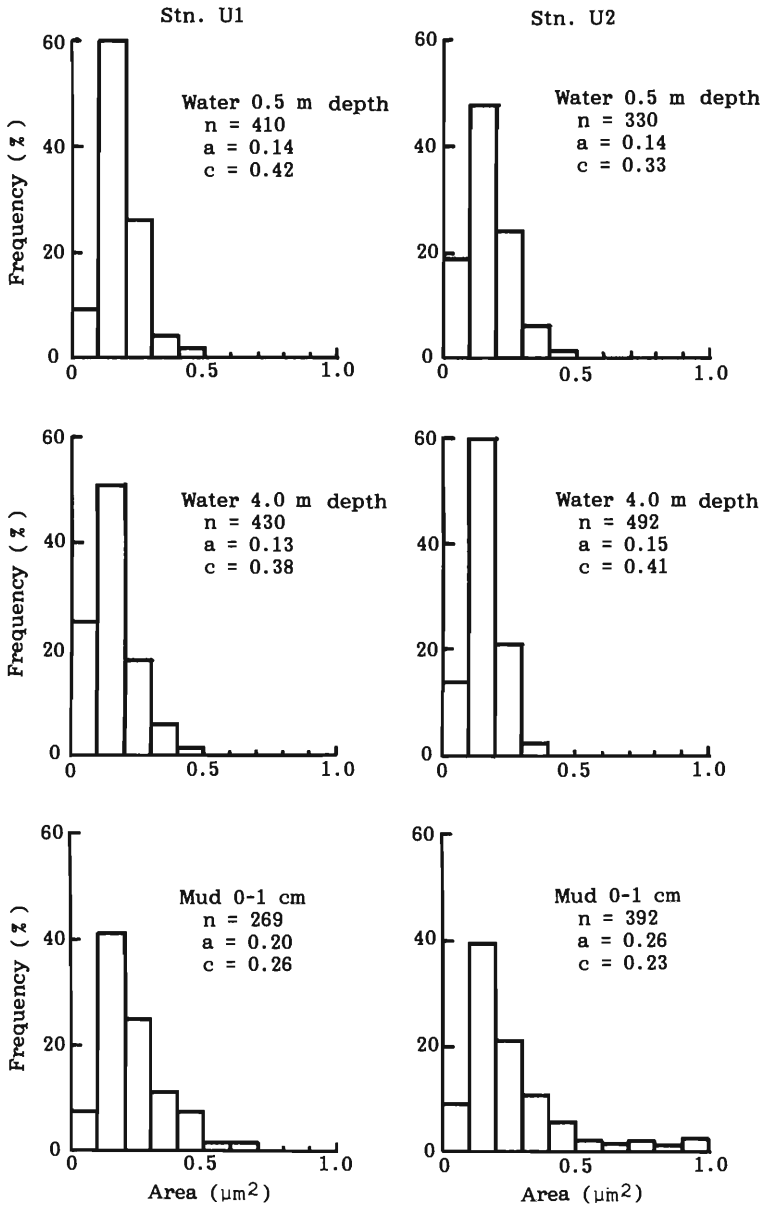

Fig. 3. Frequency distribution of cell size (μm^2) of bacteria in seawater and bottom sediments at Stns. U1 and U2 in Uragami Bay (August, 1987). n, total number of cells, the area of which was measured; a, mean cell size (μm^2); c, index of dominance.

of seawater (>5 μm). In samples of bottom sediment, the percentage of rod-shaped cells was higher than that of cocci cells.

In seawater samples from Uragami Bay, more than 50% of bacterial cells were in the size range of 0.1-0.2 μm^2 (mean, 0.14 μm^2) (Fig. 3). The area of 0.14 μm^2 is equivalent to a circle with a diameter of 0.42 μm. Bacterial cells in the range of 0.2-0.3 μm^2 accounted for about 20% of all cells investigated in seawater sampled at Stns. U1 and U2. Bacterial cells larger than 0.5 μm^2

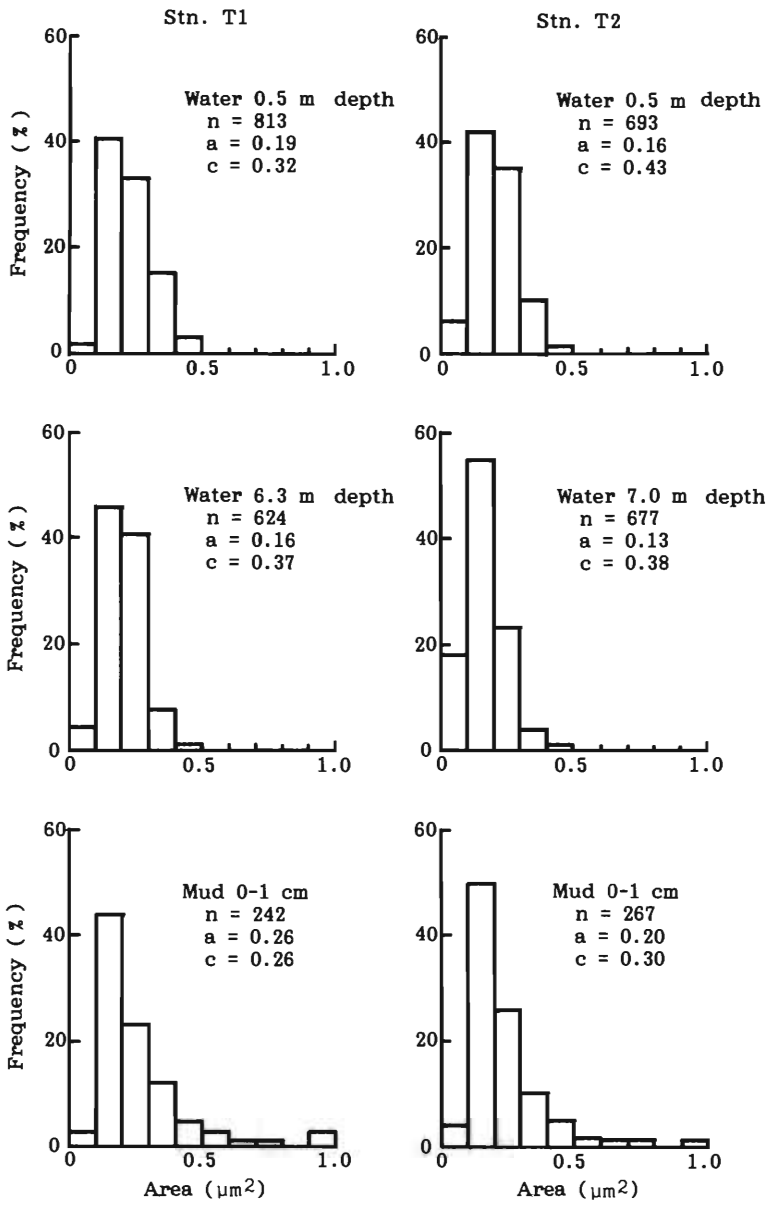

Fig. 4. Frequency distribution of cell size of bacteria in seawater, particle fraction, and bottom sediments at Stns. T1 and T2 in Tanabe Bay (July, 1987). See legend to Fig. 3 for details.

were rarely detected in any sample of whole seawater. In samples of bottom sediment, more than 10% of bacterial cells were in the range of 0.5-1.0 μm^2. The indexes of dominance (c) in whole seawater samples were in the range of 0.33-0.42 (mean, 0.38) ; these values were higher than those in bottom sediments (0.26 at Stn. U1 and 0.23 at Stn. U2).

In seawater samples at Stns. T1 and T2 (sampling depth, 0.5 m), about 40% of bacterial cells were in the size range of 0.1-0.2 μm^2 (Fig. 4). In these seawater samples, the percentage of bacterial cells in the range of 0.2-0.3 μm^2 were higher (mean, 36%) than those in Uragami Bay (mean, 22%). The pattern of size distribution in the seawater samples from Stn. T2 (depth, 7.0 m) was similar to that of samples from Uragami Bay. The mean areas of bacterial cells in seawater samples at Stns. T1 and T2 were 0.18 and 0.15 μm^2, respectively. The mean areas of bacterial cells in bottom sediments at Stns. T1 and T2 were 0.26 and 0.20 μm^2, respectively. Unlike the seawater samples, about 10% of bacterial cells in sediment samples from Stns. T1 and T2 were larger than 0.5 μm^2. This pattern was also observed in the sediment samples from Uragami Bay. The mean c in seawater from Tanabe Bay was 0.38. The values of c in samples of bottom sediment at Stns. T1 and T2 were 0.26 and 0.30, respectively.

At Stns. T1 and U1, we compared the size distributions of bacterial cells from whole seawater and particle fractions of seawater (>5 μm) (Fig. 5). In the particle fractions, the percentage of larger cells was higher. The mean cell areas in whole seawater from Stns. T1 and U1 were 0.19

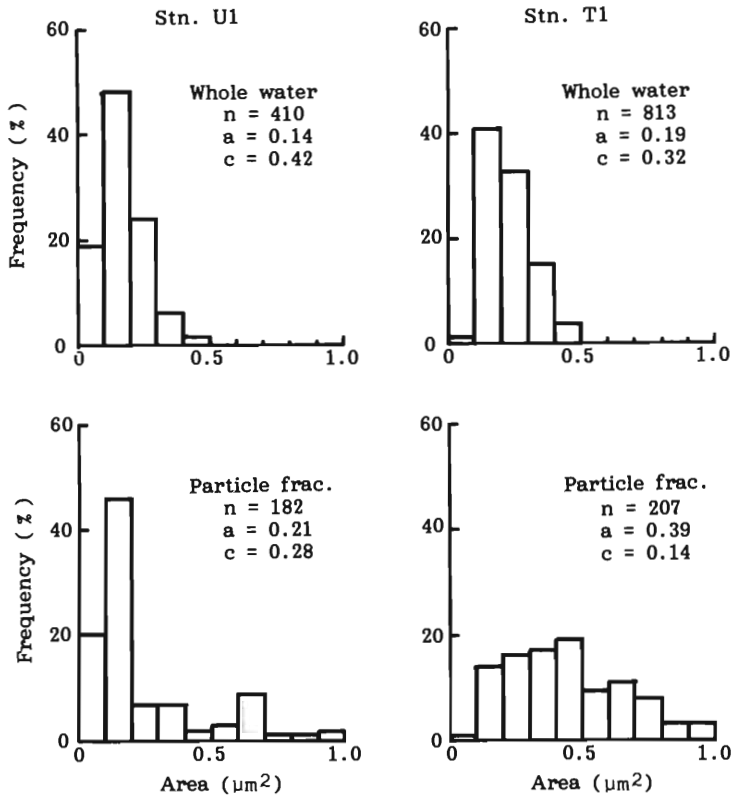

Fig. 5. Frequency distribution of cell size of bacteria in whole seawater and particle fractions (particle frac. >5 μm) at Stns. T1 and U1 (sampling depth, 0.5 m). See legend to Fig. 3 for details.

and 0.14 μm^2, and those in the fractionated water at Stns. T1 and U1 were 0.39 and 0.21 μm^2, respectively. The values of c in particle fractions at Stns. T1 and U1 were 0.14 and 0.28, respectively.

Discussion

With an epifluorescence microscopical method, Ferguson and Rublee[5] reported that 80% of bacteria in coastal seawater that they sampled was free-living, and that they were generally small cocci (diameter less than 0.5 μm). As the microscopical methods used to study cell size were different, we cannot simply compare the mean cell sizes[6]. However, a pattern similar to the one that they reported was observed in seawater samples collected at Stns. U1 and U2 in Uragami Bay and at Stn. T2 (sampling depth, 7.0 m) in Tanabe Bay. In these seawater samples, bacterial cells that were cocci and small (mean, 0.4 μm in diameter) were most common. The mean cell sizes reported for heavily eutrophic (Tokyo Bay in Japan) and oligotrophic areas (the Pacific Ocean) are in the same range, and smaller than those in mesotrophic areas (Otsuchi and Sagami Bays in Japan)[4]. Uragami Bay is not as polluted as Tokyo Bay, and the predominance of small cocci cells in seawater was probably ralated to the easy inflow of seawater from outside of the bay, which is caused by coastal upwellings around Uragami Bay[7]. Stn. T2 in Tanabe Bay was also located at a point where oceanic seawater could easily inflow. Unlike Stns. U1, U2, and T2, Stn. T1 was in a fish culturing area, where fish feeding has been done for more than 20 years and where the exchange of seawater between inside eutrophic and outside oligotrophic water was small. This may account for larger cells being more common in seawater samples from Stn. T1.

We reported previously that the composition of bacterial assemblages was different in particle fractions and whole seawater in Uragami and Tanabe Bays[1]. In whole seawater, obligate oligotrophs, which cannot grow in a nutrient-rich conventional medium (e.g. ZoBell 2216E; 3 gC/l) were most common. In particle fractions, however, almost all of the viable bacteria were facultative oligotrophs, tolerant of the concentration of organic matter. In this study, we observed a difference between free-living and attached bacteria in their cell size distribution. Free-living bacteria were small and cocci, but attached bacteria were large and rod-shaped, as Wieb and Pomeroy[8] reported. This is probably because the particles in natural seawater provide nutrient-rich microcosms and form major sites of primary production[9~11]. The heterotrophic activities in these particle fractions are higher than those in whole seawater[12,13]. The study of isolated marine bacteria (Gram-negative facultative oligotrophs) under laboratory conditions has shown that bacterial cells change in size depending on the organic concentration[14]. Large cells (about 1 μm^2) were observed at a high organic concentration (about 0.5 gC/l), but the same strains were smaller (about 0.2 μm^2) in natural seawater.

In samples of bottom sediments, the bacterial cells were generally larger than free-living bacteria, but smaller than attached bacteria in seawater. Instead of the high organic concentration in bottom sediments[1], other environmental factors, such as low pH, high C/N ratio, low redox potential, and low concentrations of dissolved oxygen, stressed bacterial cells physiologically and probably changed the composition of the bacterial assemblage. At Stn. T1 in Tanabe Bay, continuous heavy loads of organic matter allowed for eutrophic bacteria to be predominant[1]. These could only grow under nutrient-rich conditions and have been rarely detected in the natural marine environment[15,16].

In coastal areas, especially in fish-culturing inland bays like Uragami and Tanabe Bays, the water is shallow and the concentration of organic particles originating from left-over feed and fish excreta is high. This contributes to a closer relationship of the bacteria in seawater, particle fractions of seawater, and bottom sediments than in oceanic areas. In this paper, we reported a

non-random size distribution of bacterial cells in these three living sites. To understand the changes in size distributions of bacterial cells more precisely, the relationship of size to environmental factors such as organic concentration and redox potential should be studied.

Acknowledgments

We thank the late Prof. T. Harada, Prof. H. Kumai, and the staff of the Fisheries Laboratory of Kinki University for thier assitance in carrying out this study. We are also grateful to Prof. R. Tsuda of Kinki University for his kind help with the cell size measurement by the image analyzing system, and to Mr. Y. Ohnishi, Mr. N. Higuchi, and Miss Y. Taniguchi, graduates of our laboratory, for their technical assistance.

References

1) M. EGUCHI and A. KAWAI: *Nippon Suisan Gakkaishi*, **58** (1992 in press)
2) M. KIMATA, A. KAWAI, and Y. ISHIDA: *Nippon Suisan Gakkaishi*, **26**, 1227～1230 (1960)
3) E.P. ODUM: Fundamentals of Ecology (Translated from English by J. Mishima), p. 189, Baifukan, Tokyo (1974) (in Japanese)
4) M. MAEDA and N. TAGA: *La mer*, **21**, 207～210 (1983)
5) R.L. FERGUSON and P. RUBLEE: *Limnol. Oceanogr.*, **21**, 141～145 (1976)
6) J.A. FUHRMAN: *Mar. Ecol. Prog. Ser.*, **5**, 103～106 (1981)
7) R. TSUDA, H. KUMAI, and M. NAKAMURA: *Nippon Suisan Gakkaishi*, **54**, 419～428 (1988)
8) W.J. WIEBE and L.R. POMEROY: *Mem. 1st Ital. Idrobiol. Dott. Marcode Marchi*, **29**, 325～352 (1972)
9) J.D. TRENT, A.L. SHANKS, and M.W. SILVER: *Limnol. Oceanogr.*, **23**, 626～635 (1978)
10) A.L. SHANKS and J.D. TRENT: *Limnol. Oceanogr.*, **24**, 850～854 (1979)
11) G.A. KNAUER, D. HEBEL, and F. CIPRIANO: *Nature*, **300**, 630～631 (1982)
12) A.V. PALUMBO, R.L. FERGUSON, and P.A. RUBLEE: *Appl. Environ. Microbiol.*, **48**, 157～164 (1984)
13) K. FUKAMI, U. SIMIDU, and N. TAGA: *Mar. Ecol. Prog. Ser.*, **21**, 7～13 (1985)
14) M. EGUCHI: Occurrence and Survival-Strategy of Oligotrophic Bacteria in Pelagic Seawater; Doctoral thesis, pp. 86～90, Kyoto Univ., Kyoto (1990) (in Japanese)
15) Y. ISHIDA, M. EGUCHI, and K. KADOTA: *Mar. Ecol. Prog. Ser.*, **30**, 197～203 (1986)
16) M. EGUCHI and Y. ISHIDA: *FEMS Microbiol. Ecol.*, **73**, 23～30 (1990)

(Received November 6, 1991)

浦神湾ならびに田辺湾における海洋細菌のサイズ別頻度分布

江口　充・河合　章

摘　　要

内湾域の海洋細菌が生息する三つの異なる環境，すなわち海水，海水中の懸濁物（サイズ5 μm 以上），海底堆積物における海洋細菌のサイズ別頻度分布を，浦神湾ならびに田辺湾を対象として調査した．細菌細胞のサイズの測定は，走査型電子顕微鏡と画像処理装置を用いて行い，細胞の面積（μm^2）で示した．海水中の細胞の形態は，浦神湾ならびに田辺湾ともに類似した傾向を示し，球菌状であったが，細胞サイズは少し異なり，浦神湾では平均面積が0.14 μm^2，田辺湾では0.17 μm^2 であった．海水懸濁物（付着細菌群）では，海水中の浮遊細菌群に比べて，大きい桿菌状の細胞（平均面積0.30 μm^2）の全細胞中に占める割合が高くなり，優占度指数は低くなった．また，底泥堆積物では，比較的桿菌状の細胞が多く，細胞サイズは海水全体の細胞サイズと懸濁物中の細胞サイズの中間的な値を示した．