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Vertical Succession of Attached Bacteria and Its Relationship to the Distribution of Organic Matter in Seawater

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Abstract: Vertical change of the heterotrophic bacterial community attached to particulate organic matter (POM) was studied in relation to the distribution of POM in the neritic sea of Japan. The analysis of bacterial community was performed by dividing the isolates into five groups: *Vibrio* (V), *Pseudomonas-Alcaligenes* (Ps), *Acinetobacter-Moraxella* (Ac), Chromogenic (C) and Gram-positive (Po) groups. The community structure of attached bacteria was quite different from that of free-living in the ambient seawater. In the water column, the community of attached bacteria showed the distinct vertical succession, which was: Ps→V→(Ps+Ac)→(Ac+C)→Ps. The attached bacteria in the layers where the decomposition of POM proceeded actively had high substrate-decomposing activity, whereas in the deeper layers with limited supply of the "fresh" POM the activity was low. These results suggest that the community of attached bacteria showed the vertical succession with the proceeding of POM decomposition from the community with a high biochemical activity to a low activity, as associated with the change of genera.

Key words: attached bacteria, succession, decomposition, particulate organic matter

Introduction

In marine ecosystems, heterotrophic bacteria contribute significantly to the decomposition of organic matter and the regeneration of inorganic nutrients. The behaviour of the members of their community in these decomposition process, however, has not been fully clarified as yet. Comparing with the studies on their quantitative distribution, those on qualitative aspects, structure and activities of bacterial community, are scarce. There has been little information on the evaluation of factors which control the development and fluctuation of bacterial flora in natural sea environment. Simidu et al. (1977) attributed the formation of specific bacterial flora in the eutrophic Tokyo Bay to the antagonistic effects of phytoplankton. However, this type of studies has not yet been made in the open ocean.

Recently, Fukami et al. (1981; 1985b) investigated the change of bacterial community attached to the particulate matter during the decomposition process of phyto- and zooplankton organic matter in the laboratory. In these papers, they reported that the bacterial community showed successional changes with the proceeding of decomposition of phytoplanktonic particulate organic matter (POM) in terms of both generic composition and biochemical activities. The sequency of successional change was as follow: *Pseudomonas-Alcaligenes* group with protein-hydrolyzing ability (Ps 1 group)→ *Acinetobacter-Moraxella*(Ac) group → Chromogenic (C) group → *Pseudomonas-Alcaligenes* group with no protein-hydrolyzing ability (Ps2 group). In addition, the community changed from that with the high decomposing ability of macromolecular organic substrates to

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that of the low ability. During the decomposition of zooplankton, the *Vibrio* (V) group of bacteria predominated at first and the change was as follow: V→Ps 1→Ac→C→Ps 2 (Fukami et al., 1985b). These results lead us to speculate that the formation of a specific bacterial flora depends, to some extent, on the chemical composition of organic matter in seawater.

In the marine environment, POM is produced by the photosynthesis of phytoplankton in the upper euphotic layers and is transported downward with gradual change of chemical composition by microbial decomposition. This implies a vertical distribution of POM in the different stages of decomposition process or of POM of different chemical compositions. If the succession of bacterial communities observed in the process of decomposition in the laboratory experiments (Fukami et al., 1981; 1985b) are general phenomena, some vertical change of the communities of heterotrophic bacteria will be expected in water column.

In this study, the vertical changes of the community of heterotrophic bacteria are investigated in neritic sea area of Japan and its relationship to the chemical composition of POM is examined.

Materials and Methods

Seawater Sampling

Seawater samples were collected vertically from Sagami Bay (Stn. A-1, 35°05′N, 139°20′E) during the KT-78-13 cruise of R/V Tansei-Maru, Ocean Research Institute, University of Tokyo. Seawater samples for the microbiological analyses were collected with sterile ORIT samplers (Taga, 1968) or sterile Niskin butterfly water samplers. For the chemical analyses, seawater samples were taken with 25L Van Dorn samplers.

Microbiological analyses

Numbers of viable heterotrophic bacteria (V.C.) in untreated raw seawater samples were determined using duplicate plates of PPES-II medium (Taga, 1968). After bacterial cells in a water sample were condensed onto a sterile Nuclepore filter (pore size 0.2 μm), the filter was placed aseptically on the plate medium with sterile forceps for the incubation. The bacteria making colonies on plate media in these raw seawater samples were referred to as "colony-forming bacteria in untreated seawater (CFB-US)". For counting the number of attached bacteria, subsamples of seawater were filtered through a sterile Millipore filter (pore size 5 μm), and then filter was homogenized by a sterile blender with 30 ml sterile filtered seawater containing 5 ppm of Tween 80 to disperse the attached or clumped bacteria (Jones and Jannasch, 1959). The bacterial suspensions obtained as above were inoculated on plates of the same media. The bacteria making colonies on plate media obtained as above were referred to as "colony-forming bacteria on particle (CFB-P)". The incubation of inoculated plates of both CFB-US and CFB-P were carried at 20°C for 2 weeks. Plates on which more than about 30 up to 300 colonies appeared were used to determine the numbers of CFB-US and CFB-P. For the vertical profile studies, 20 strains of bacteria were isolated at random from each cultured plate at each depth. After purification, isolated bacteria were classified by the scheme of Fukami et al. (1981) into 5 groups; *Pseudomonas-Alcaligenes* (Ps) group, *Acinetobacter-Moraxella* (Ac) group, *Vibrio* (V) group, Chromogenic (C) group and the Gram positive (Po) group. Several tests were also performed to examine biochemical activities of those isolates, for the hydrolysis of gelatin (Gel.), starch (St.), tributylin (Tr.) and chitin (Chi.). The detailed procedure was described in a previous paper (Fukami et al., 1981).

Microscopic determination of bacterial and diatom numbers

Total number of bacteria was counted by acridine orange direct count method (Fukami et al., 1983). In addition, the enumeration of phytoplankton was performed at Stn. A-1 in Sagami Bay. Seawater samples collected vertically were

filtered onto a Nuclepore filter (pore size 0.2 μm) and were observed at 400× magnification using an epifluorescence microscope. Only plankton cells obviously identified as diatoms were counted.

Chemical analyses

The dry weight of particulate matter (ss) was determined by the method of Strickland and Parsons (1972). Subsamples of seawater were filtered through preweighed Reeve Angel 984H glass fiber filters. After filtration, the filters were rinsed with a small portion of filtered distilled water quickly, dried at 70°C and weighed again. The concentrations of particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined by the method of Sharp (1974) using the CHN-corder (Yanagimoto, MT-2). Dissolved organic carbon (DOC) concentration was determined by the method of infrared gas analysis (Menzel and Vaccaro, 1964). The amount of particulate protein condensed onto the same type of glass fiber filters were determined by the method of Iwamura et al. (1970). Detailed procedures were described in a previous paper (Fukami et al., 1985a). Chlorophyll a (Chl. a) contents were determined spectrophotometrically after SCOR/UNESCO (1966) and/or Lorenzen (1967).

Results

Vertical distribution of POM at Stn. A-1 of Sagami Bay during the cruise of KT-78-13 is shown in Fig. 1. A clear thermocline was formed between 20 and 60 m depth. The dry weight of particulate materials (ss), the concentrations of POC, PON and particulate protein showed maximum values in the surface layer. Although the chlorophyll a maximum layer was observed at 40

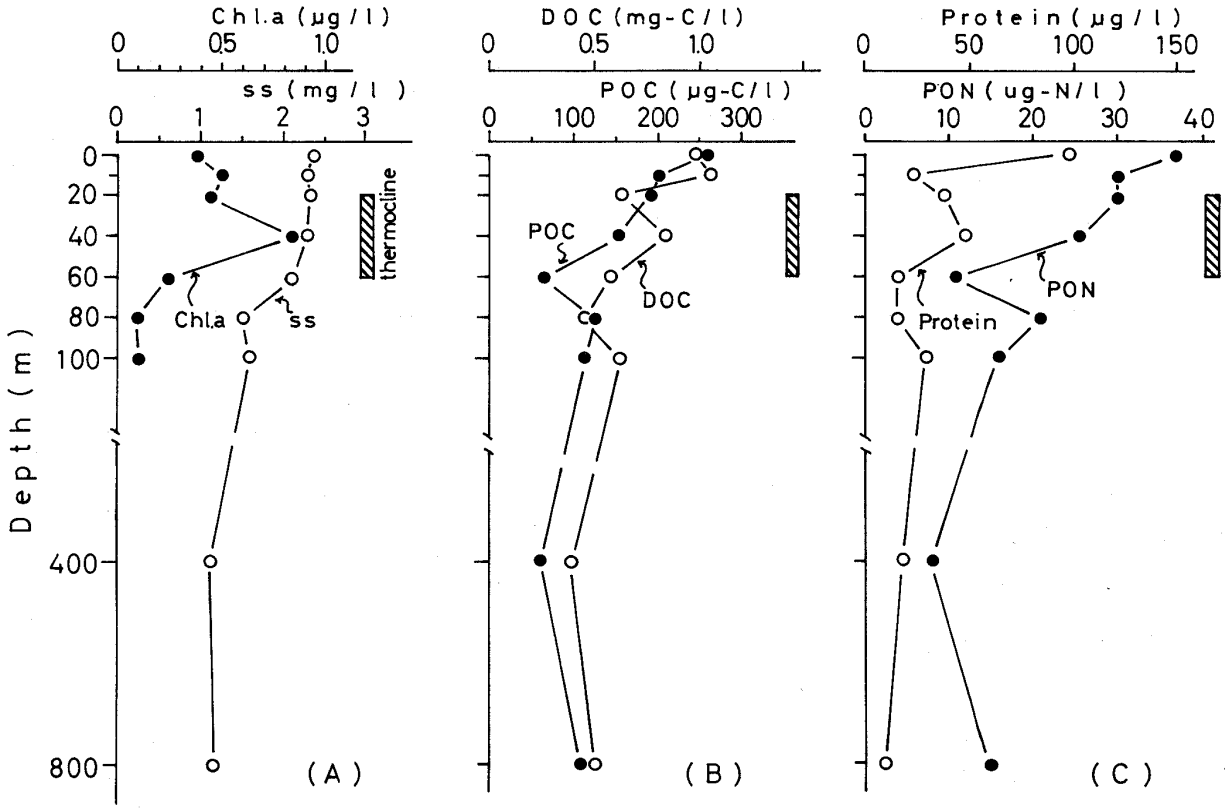

Fig. 1. Vertical profiles of several chemical parameters at Stn. A-1 in Sagami Bay during the KT-78-13 cruise. The thermocline developed between 20 and 60 m depth (indicated as shadow column). Abbreviations are as follows: (A)ss: weight of seston, Chl. a: chlorophyll a, (B)POC: particulate organic carbon, DOC: dissolved organic carbon, (C)PON: particulate organic nitrogen, Protein: particulate protein.

m depth, the cell number of diatom was high in layers shallower than 20 m (Table 1). The concentration of POC and particulate protein decreased sharply and DOC concentration increased from surface to 10 m (Fig. 1). In chlorophyll a maximum layer at 40 m depth, although both ss and POC did not give any peak, particulate protein showed the second peak (Fig. 1). Fig. 2 shows the vertical fluctuation of Chl. a and pheopigments obtained by the method of Lorenzen (1967). At 20 m depth, the concentration of pheopigments exceeded the Chl. a and the POC concentration decreased sharply (Figs. 1 and 2). Below 40 m layer with the maximum Chl. a concentration, the concentration of particulate protein decreased rapidly again and pheopigments exceeded the Chl. a (Figs. 1 and 2). These results suggest that the particulate organic matter with high protein content, which may due to the production of phytoplankton, was accumulated at the surface and 40 m depth layers. The organic matter in the surface layer, however, was decomposed rapidly down to the depth of 20 m. The POM at 40 m layer was also decomposed beneath this layer, and in the 20 m and deeper water layers particulate matter would consist of rather old and refractory organic matter.

The colony-forming bacteria in untreated raw seawater (CFB-US) gave the maximum count at 0 m layer and gradually decreased as the depth increased (Fig. 3). The numbers of CFB were about 0.1% of total bacterial numbers obtained by direct counting method (data not illustrated). The vertical change of the community structure of CFB is shown in Fig. 4. In the CFB-US bacteria of V group occurred in almost all layers throughout the water column, except in the surface layer. The chromogenic bacteria (C group) predominated in subsurface and intermediate layers between 80 and 400 m depth. The proportion of Ps group

Table 1. Vertical distribution of diatom at Stn. A-1 in Sagami Bay during the KT-78-13 cruise

Depth (m)	Diatom (cells/ml)
0	295
10	322
20	263
40	42
60	13
80	10
100	0

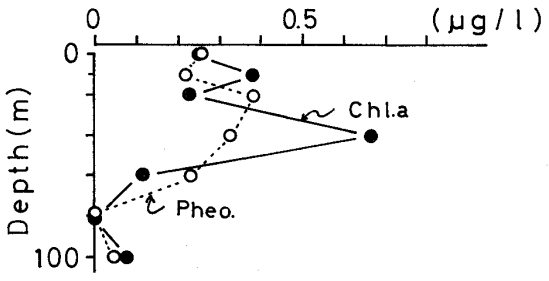

Fig. 2. Vertical profiles of Chl. a and pheopigments at Stn. A-1 in Sagami Bay during the KT-78-13 cruise.

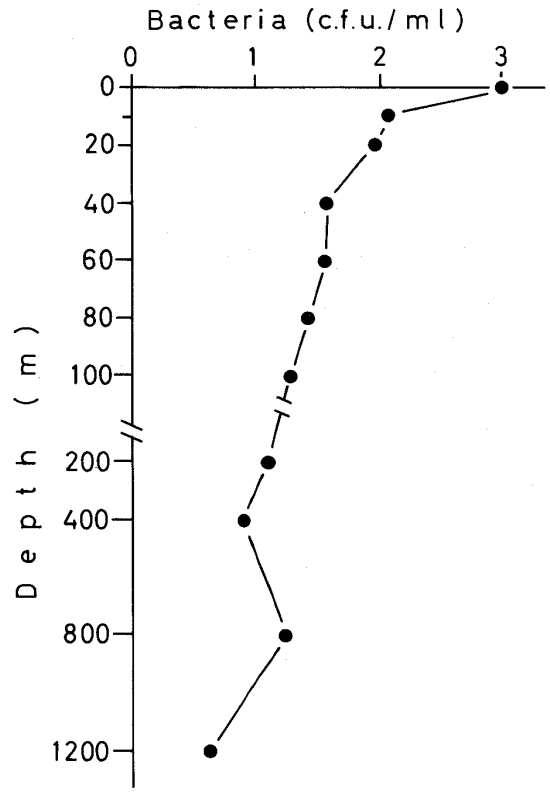

Fig. 3. Vertical distribution of colony-forming bacteria in untreated raw seawater (CFB-US) at Stn. A-1 in Sagami Bay during the KT-78-13 cruise.

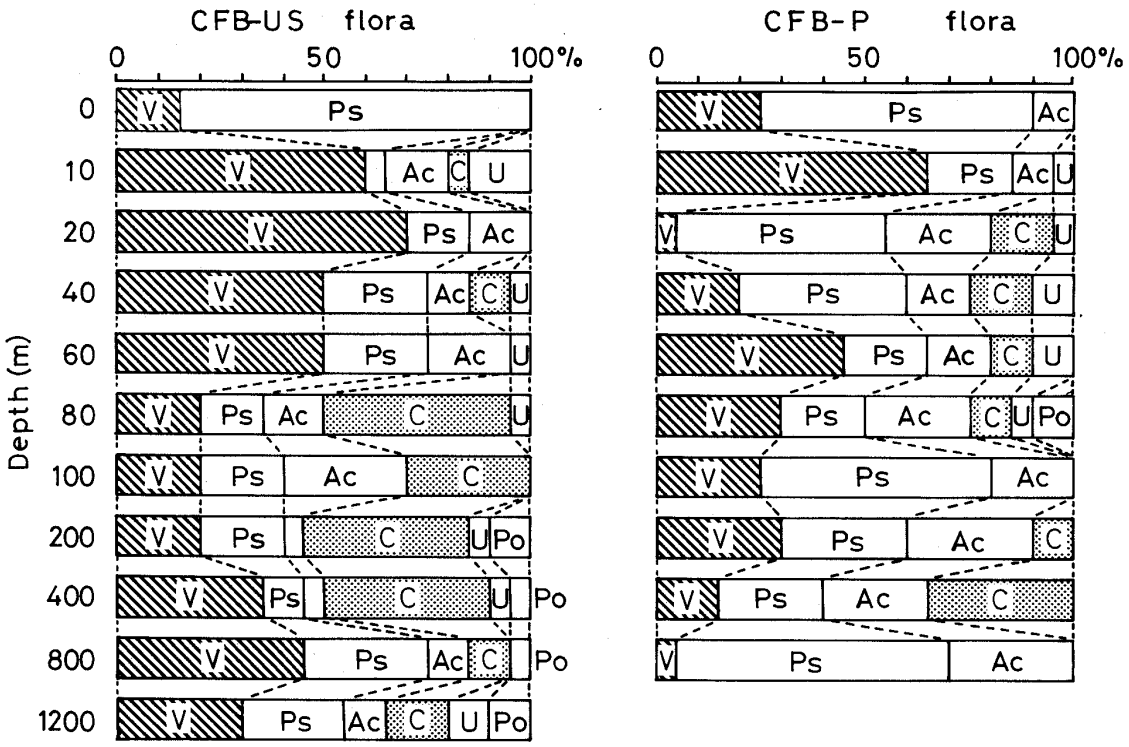

Fig. 4. Vertical fluctuation of bacterial community structure at Stn. A-1 in Sagami Bay during the KT-78-13 cruise. (left) the colony-forming bacteria in untreated raw seawater (CFB-US), (right) the colony-forming bacteria on particle (CFB-P). See text for the symbols.

in total colony number of CFB-US was rather low throughout the water column except in the surface layer. On the other hand, the percentage of V group in the colony-forming bacteria attached on particle (CFB-P) gave two obvious peaks at 10 and 60 m and was very low at 20 m where the contribution of V group in the CFB-US was the largest. The proportion of V group in CFB-P decreased with depth from 60 m down to 800 m. The abundance of the chromogenic bacteria (C group) was relatively low throughout the water column. The bacteria of Ps group, on the other hand, occurred in greater percentages in the CFB-P community. Although the community of CFB-US included some of CFB-P, the community structure of CFB-P was different from CFB-US, suggesting that the bacteria attached to particulate matter was quite different from that of free-living in the ambient seawater. The community structure of CFB was variable from depth to depth, and in the community of CFB-P, two vertical successive changes were observed ; the first was from the surface to 20 m, and the second was from 40 to 800 m. To clarify the vertical changes of bacterial community in more detail, potential decomposition abilities of gelatin, starch, tributylin and chitin were tested for isolated bacteria from each depth. Results are shown in Table 2 as the percent occurrence of substrate-decomposing bacteria. In four substrates (gelatin, starch, tributylin, chitin), occurrence rate of tributylin-decomposing bacteria was the highest, with the average of CFB-P for each depth of 66%, then followed to those of gelatin, starch and finally chitin. There were no significant differences of the average occurrence rate values of each substrate between CFB-US and CFB-P (Table 2). To know the vertical change of CFB-P community, a tentative criterium of "Decomposition Index (D.I.)" was used, which was the summation of the occurrence rates of bacteria for four substrates. This index, therefore, has a minimum 0 and a maximum of 400. If the D.I. value is high, the community of CFB would have high potential decomposing ability of these

Table 2. Percent occurrences of the substrate-decomposing bacteria to total (CFB-US) or attached (CFB-P) bacteria isolated from various depths at Stn. A-1 in Sagami Bay during the KT-78-13 cruise. Decomposition Index represents the summation of the percent occurrences of four substrates.

Substrate	Starch (%)		Gelatin (%)		Tributylin (%)		Chitin (%)		Decomposition Index	
Depth (m)	total	attached	total	attached	total	attached	total	attached	total	attached
0	70	90	65	70	70	70	15	35	220	265
10	35	65	60	65	50	85	70	75	215	290
20	65	10	75	5	60	55	75	10	275	80
40	45	20	45	35	45	30	45	15	180	100
60	45	40	45	45	65	80	50	50	205	215
80	15	25	5	50	15	75	20	30	55	180
100	10	30	10	60	45	85	20	30	85	205
200	25	25	20	45	50	55	20	30	115	155
400	0	25	15	20	50	70	30	10	95	125
800	35	5	45	25	85	50	45	5	210	85
1200	15		15		30		35		95	
*average	35	34	39	42	54	66	39	29	166	170

*not contained the value of 1200 m depth

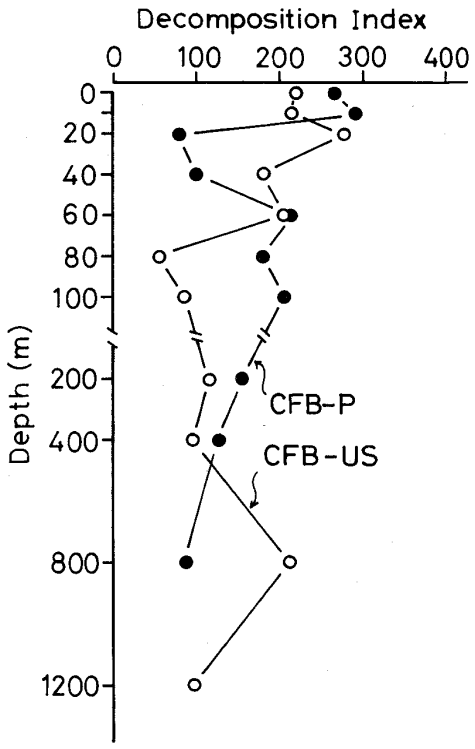

Fig. 5. Vertical fluctuation of Decomposition Index (D.I.), which represents the summation of the percent occurrences of substrate-decomposing bacteria. See text for the details.

four macromolecular substrates. As shown in Table 2 and Fig. 5, the D.I. for CFB-P was high at both 0 and 10 m, decreased sharply at 20 and 40 m. However, the value increased again at 60 m and was followed by gradual decrease with depth down to 800 m. The vertical profile of D.I. value in CFB-P were accompanied with the vertical change of the CFB-P community structure as shown in Fig. 4. The Ps group dominant at 20 and 800 m had little ability to decompose four kinds of organic matter given as a substrate, being different from that in the surface and 100 m layers.

Discussion

In the present study, the vertical change of the community of heterotrophic bacteria was studied on the colony-forming bacteria (CFB) grown on plate media. Recently there are many criticisms that the CFB are not the representative of bacterial community as they comprise only 0.01 to 0.1% of total community of aquatic bacteria, especially in the oligotrophic water (i.e. van Es and Meyer-Reil,

1982). In our study, the proportion of CFB was more or less 0.1% of total bacterial numbers obtained by acridine orange direct count method with epifluorescence microscope. Although there is such a methodological limitation of the plate count technique, we dared employ this technique to analyze bacterial community structure because we wished to know qualitative aspect of microbial ecology and find out a process of the formation of specific flora in the natural waters. In the future when the methodology develops we will certainly get the precise information on the flora by such as immunofluorescence.

Sagami Bay, investigated in this paper, is a relatively oligotrophic water body in spite of its short distance from the mainland of Japan because of incomming of oligotrophic Kuroshio current. As shown in Fig. 1, there distributed the high concentration of particulate protein at the surface and the chlorophyll a maximum layer of 40 m depth. From the result of our laboratory experiments (Fukami et al., 1985a) that POM was of relatively high protein content in the early stage of decomposition process, it is assumed that most POM of surface and 40 m depth were still in the early step of decomposition process and it can be said "fresh" POM. Vertical distribution of several chemical parameters illustrated in Fig. 1 shows that the POM was gradually decomposed in process of sinking downward from the surface layer to the depth of 20 m and there remained at 20 m an "old" POM, which means the one whose decomposition step was rather proceeding. Another "fresh" POM with high protein content at 40 m was decomposed beneath this layer, and in the deep water particulate matter consisted of rather "old" and refractory organics.

In the CFB-P, two distinct vertical successions of bacterial community were observed from the surface to 20 m and from 40 m to 800 m (Fig. 4). The sequences of the vertical succession with depth were as follows: in the uppermost layer Ps group predominated, then V group became dominant, next the dominant group was changed to Ps+Ac, then to Ac+C and finally another type of Ps group predominated. This Ps group with little biochemical activity seemed to be the same group as "Ps 2 group" in the laboratory experiment (Fukami et al., 1981, see Introduction). The beginning layers of the succession of CFB-P community coincided with those where fresh POM distributed with high concentration in the water column, and from these layers the community structure changed downward. The results presented in Table 2 and Fig. 5 show that the CFB-P communities had high decomposing ability (with high D.I. value) in the layers where the POM would be decomposed actively and had low ability in the 20 m and below 400 m layers. These results strongly suggest that during the process of decomposition of POM the bacterial community attached on particulate material shows the successional change in terms of both generic composition and its biochemical activity in the natural environment. In this case, as the depth can be transformed to the time scale, the various stages of the decomposition process may expanded vertically.

Now for converting the depth to the time scale, we must consider the sinking rate of POM. There are many reports on the sinking rate of particulate matter. The sinking rate of living phytoplankton, which was measured in the sinking chamber in the laboratory, was generally very low: usually less than 1 m/day (Smayda, 1974; Bienfang, 1981), whereas the cell of dead phytoplankton sank faster than living one, in some cases about 3.8 fold higher (Smayda, 1974). According to Burns and Rosa (1980), the settling velocity of detrital organic matter with 10-64 μm size range was about 1.54 m/day. The fecal pellets of zooplankton sank much faster than the cells of phytoplankton (i.e. Small et al., 1979). The velocity depends, of course, on the size of the pellet. Sinking rate of fecal pellets ranged from 36 m/day for the minimum value of 53×106 μm size to 376 m/day for the maximum value of 173×260 μm size pellet, usually around 100 m/day (Smayda, 1969).

POM in the water column consists of various

types of organic materials, such as living phytoplankton, zooplankton, detritus, fecal pellet and others. Sinking rate of decomposing organic matter composed mainly of the detritus of phytoplankton origin can be regarded as around 2 m/day. On the other hand, fast settling organic matter such as large fecal pellets of zooplankton are scarce in natural sea water (McCave, 1975), and it is supposed that the POM taken by Van Dorn sampler contains relatively small size pellets. Thus the sinking rate of the small size pellet may be estimated several tens meter per day.

In the laboratory experiments, the active process of POM decomposition lasted for about 2 weeks (Fukami et al., 1981). If the active process of decomposition in the water column is also assumed as about 2 weeks, a sinking rate of POM of surface layer in this study must be approximately 1.5-2 m/day until settling down to 20 m depth with being decomposed. This value is well consistent with the settling velocity of dead phytoplankton. This suggests that POM from surface to 20 m layer consists mainly of the decomposing detritus of phytoplankton origin. Also below 40 m, the bacterial community showed one cycle of successional change down to 800 m. Considering that decomposition rate of POM would decrease due to the low temperature, POM of the 40 m layer must be gradually decomposed while sinking down to 800 m layer. Thus, the settling velocity of POM of 40 m was assumed as about thirty or forty meters per day. This implies that POM below 40 m layer would consist of some small fecal pellets or detritus of zooplankton rather than that of phytoplankton.

From the results of the present study, it is suggested that by investigating the community structure of microorganisms attached to POM we can guess the origin and the decomposition step or chemical composition of POM in seawater.

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