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Microbial decomposition of phyto- and zooplankton in seawater. II. Changes in the bacterial community

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ABSTRACT: Fluctuation of bacterial communities during decomposition of the diatom *Skeletonema costatum* (Grev.) Cleve, and the copepod *Tigriopus japonicus* Mori was investigated under laboratory conditions. A 5.0 μm Nuclepore filter was employed to separate the 'attached' community from a 'free-living' one. During phytoplankton decomposition, the attached community changed as follows: *Pseudomonas-Alcaligenes* (Ps) group → *Acinetobacter-Moraxella* (Ac) group → Chromogenic (C) group → Ps• (Ps group with low growth rate). During zooplankton decomposition, on the other hand, the *Vibrio* (V) group of bacteria predominated at first and the change was as follows: V → Ps → Ac → C → Ps•. During the later stages of phytoplankton decomposition, fresh plankton cell material was added again to the system. The results showed that the Ps fraction of bacteria appeared again as the dominant community and that a similar succession could be traced in the bacterial community. In addition, high per-cell specific activity (maximum assimilation velocity/total cell count number) of attached bacteria, observed during the earlier stages of decomposition, gradually decreased to as low as that of free-living bacteria as the decomposition proceeded. These results suggest that during the process of decomposition of particulate organic matter the bacterial community involved in the degradation shows a successive change not only in generic composition but also in terms of the heterotrophic activity of the bacteria in the community.

INTRODUCTION

Although heterotrophic bacteria occupy a significant position in the marine ecosystem, the roles of various bacterial groups in ecological processes have not been fully clarified. Recently, as an approach to the study of the circulation of organic matter by bacteria, several workers divided the community of marine bacteria into 2 groups: bacteria associated with particulate materials (attached bacteria) and bacteria in the planktonic state (free-living bacteria) (Paerl, 1973; Goulder, 1977). Fukami et al. (1983a) investigated the fluctuation of the number of bacteria during decomposition of a phytoplankton bloom and reported that only the number of attached bacteria increased as decomposition proceeded, while that of free-living bacteria remained almost constant. In field studies, several reports have indicated that the density of attached bacteria fluctuates with the concentration of suspended solids (Goulder, 1976), or particulate organic carbon (POC) (Fukami et al., 1983b), whereas the density of free-living bacteria showed neither significant geographical, vertical or seasonal fluctuations nor a relation to the concentration of suspended solids (Wilson and Stevenson, 1980).

There is some possibility, of course, that some of the free-living bacteria become attached to particulate organic matter (POM) and then grow on the POM when the concentration of detrital particles increases. On the other hand, differences between attached and free-living bacteria have been found by many workers both in cell size (Wiebe and Pomeroy, 1972; Ferguson and Rublee, 1976), and in rate of assimilation of organic substrates (Hodson et al., 1981; Paerl and Merkel, 1982). The differences in size and activities between these 2 communities are not constant; sometimes the differences reach more than 1 order of magnitude, while in other cases they are almost equivalent. These results led us to suggest that the communities of attached bacteria may fluctuate both in composition and activity according to the conditions under which they live.

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Fukami et al. (1981) reported differences between communities of attached and free-living bacteria with special reference to the bacterial flora and the potential ability of bacteria to decompose organic substrates. They also indicated that the community of attached bacteria showed a successional change during phytoplankton decomposition in laboratory experiments.

The present study was designed to examine further the fluctuation of bacterial communities during decomposition of both phyto- and zooplankton in seawater. Changes in assimilation activity were also studied. Changes in organic fractions during decomposition were reported in the preceding paper (Fukami et al., 1985). In this paper, we report changes in the composition of the bacterial community and in bacterial activities.

MATERIALS AND METHODS

The diatom *Skeletonema costatum* (Grev.) Cleve and the copepod *Tigriopus japonicus* Mori were used for our experiments. Experimental system, preparation of decomposed plankton and inoculated microorganisms are the same as those described previously (Fukami et al., 1985).

Enumeration of bacteria. Bacterial numbers were counted by both the spread-plate method using Medium 2216E (Oppenheimer and ZoBell, 1952) and the direct microscopic method using an epifluorescence microscope (Fukami et al., 1983b). The bacterial count was performed for both attached and free-living bacteria. A sterile 5.0 µm Nuclepore filter was used to separate attached and free-living bacteria. The procedure is illustrated in Fig. 1 and described in detail by Fukami et al. (1981).

Classification of the isolated bacteria. About 20 strains of bacteria were isolated at random from each plate. The colonies were isolated as follows: a line is drawn freely on a plate, and colonies along the line are picked up, or the plate area is fractionated into several subareas and all colonies included in one subarea are picked up. After purification, isolated bacteria were classified into 5 groups (Fukami et al., 1981): *Pseudomonas-Alcaligenes* (Ps) group, *Acinetobacter-Moraxella* (Ac) group, *Vibrio* (V) group, chromogenic (C) group, Gram-positive (Po) group.

Determination of heterotrophic activity. Cell suspensions containing attached or free-living bacteria (prepared as in Fig. 1) were employed for studies of heterotrophic assimilation activity. The method used is based on the technique of Wright and Hobbie (1966). The procedure is illustrated in Fig. 1. Five different concentrations of uniformly labelled ^{14}C-amino acid mixture (RCC Amersham, 100 µCi = 47.57 µg amino acid = 21.22 µg C) (0.01, 0.05, 0.1, 0.2, 0.5 µCi/0.5 ml) were added to 2 ml samples. Composition of the amino acid mixture (by radioactivity) was: L-alanine 9.5 %, L-arginine 7.0 %, L-aspartic acid 10.0 %, L-glutamic acid 9.0 %, glycine 6.0 %, L-histidine 1.5 %, L-isoleucine 6.0 %, L-leucine 12.5 %, L-lysine 5.0 %, L-phenylalanine 7.5 %, L-proline 5.5 %, L-serine 3.0 %, L-threonine 5.5 %, L-tyrosine 6.0 %, L-valine 6.0 %.

Fixed samples for the blank were prepared by adding filtered neutral formalin (final concentration 5.0 %) and were also added to each concentration of the labelled substrate. Samples and blanks were incubated in a water bath at 20°C. Incubation time was fixed at 20 min since a linear assimilation curve was obtained up until a time of 20 to 30 min in a preliminary experiment. After incubation, microbial activity was stopped by filtering onto a Millipore GS filter (pore size 0.22 µm). To minimize loss of labelled intracellular pool material, a fixer was not used (Azam and Holm-Hansen, 1973; Griffiths et al., 1974). The filter was washed 4 times with ice-cold filtered seawater, then placed in a counting vial and dried under an infrared lamp. After addition of 5 ml of scintillation fluor solution (Filter Count, Packard), radioactivity was measured in an LKB Wallac 1215 liquid scintillation counter. Parameters of maximum uptake velocity (V_{max}), turn-over time (Tt), and the saturation constant

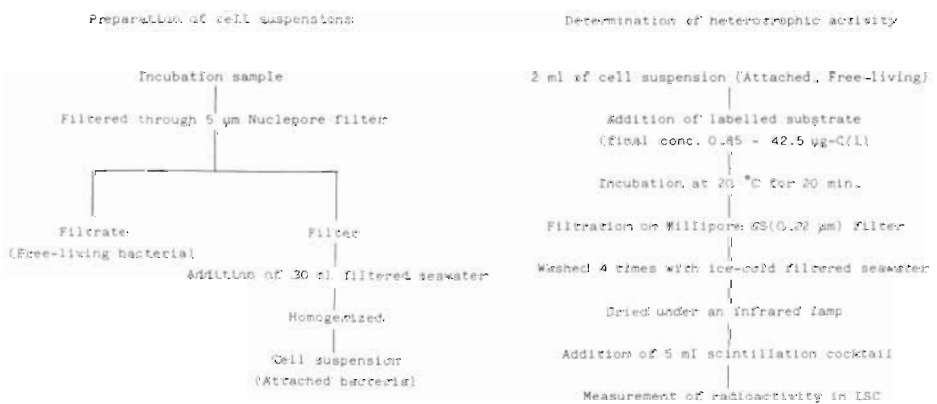

Fig. 1. Flow diagram for preparation of cell suspensions (left) and experimental procedure for activity analysis (right)

plus the amount of substrate present naturally (Kt + Sn) were calculated using the Lineweaver-Burke equation.

RESULTS

Changes in the viable plate count of bacteria during decomposition of *Skeletonema costatum* are illustrated in Fig. 2; results of the experiment with *Tigriopus japonicus* are shown in Fig. 3 (total count) and Fig. 4 (viable plate count).

Both direct and viable plate counts revealed similar fluctuation patterns for both phyto- and zooplankton samples. The number of bacteria increased rapidly on the first day. In the first few days, the count of free-living bacteria was higher than that of attached bacteria. The number of free-living bacteria then decreased rapidly, while that of attached bacteria remained high until Day 20. In the experiment using *Skeletonema costatum*, after addition of fresh cell material on Day 23, a similar fluctuation of bacterial numbers was observed (Fig. 2).

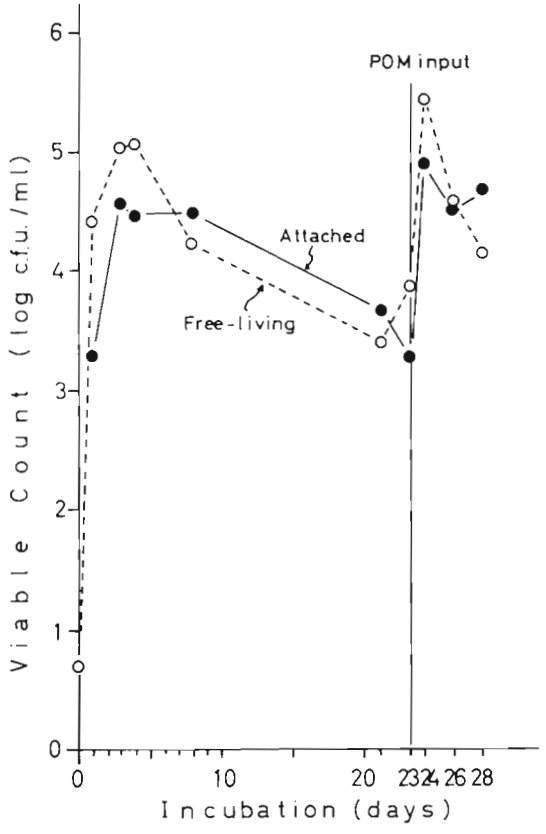

Fig. 2. Fluctuation in the number of attached and free-living bacteria obtained by viable plate count during decomposition of *Skeletonema costatum*. On Day 23, fresh plankton material was added to the system

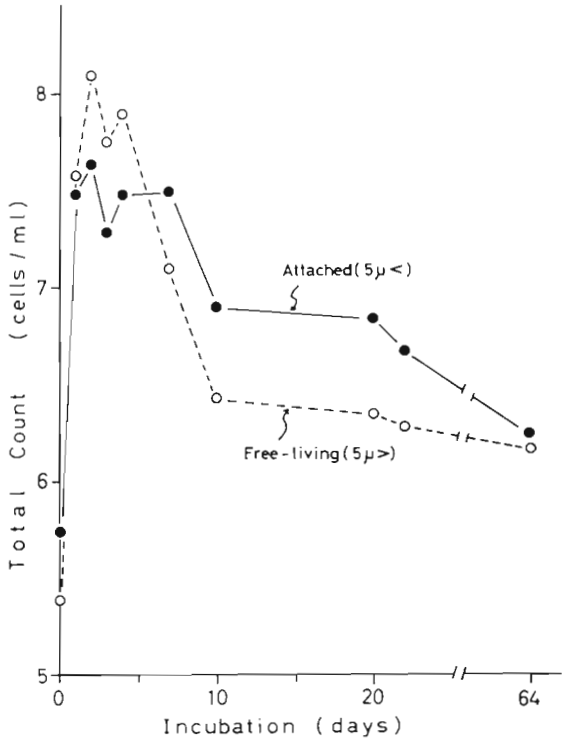

Fig. 3. Fluctuation in the number of attached and free-living bacteria obtained by total direct count during decomposition of *Tigriopus japonicus*

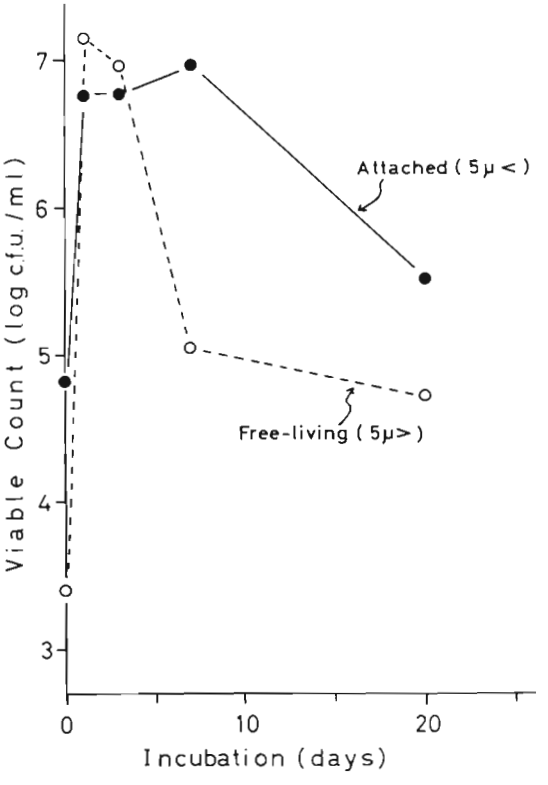

Fig. 4. Fluctuation in the number of attached and free-living bacteria obtained by viable plate count during decomposition of *Tigriopus japonicus*

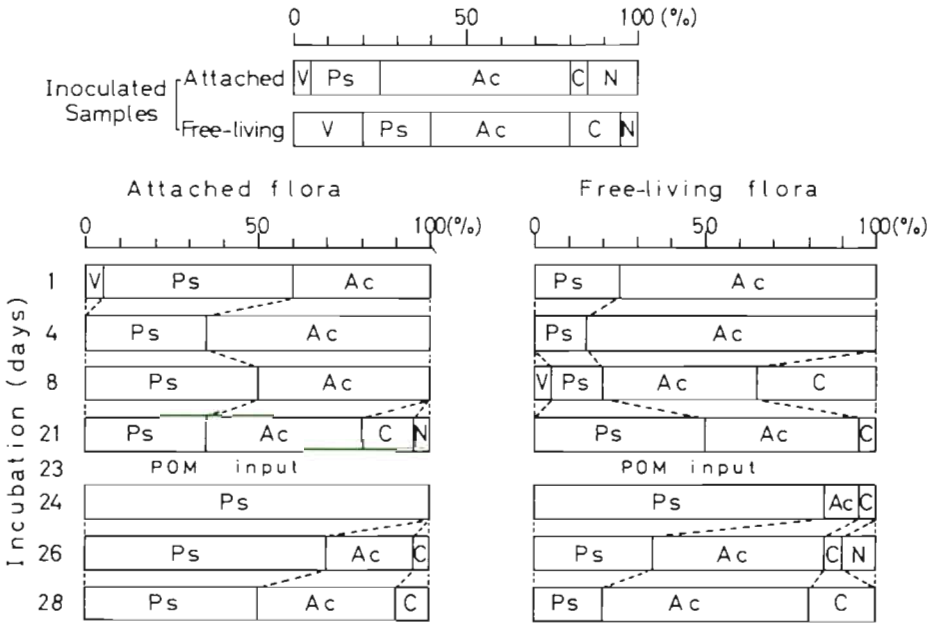

Fig. 5. Fluctuation in bacterial flora during decomposition of *Skeletonema costatum*. On Day 23, fresh plankton material was added to the system. See text for explanation of symbols

Changes in composition of the bacterial flora are given in Fig. 5 and 6. During several decomposition experiments, although the percentages of each bacterial group fluctuated to some extent, the following general changes in the flora were observed. In the experiment using *Skeletonema costatum*, while various kinds of bacteria were observed in the inoculated samples, the Ps group in the attached community and the Ac group in the free-living community predominated on the first day (Fig. 5). The proportion of the Ps group in the attached community decreased gradually and the percentage of the Ac and the C groups increased until Day 21. After addition of fresh cell material on Day 23, however, the bacteria of the Ps group became again dominant (Fig. 5). In the decomposition of zooplankton, the bacteria of the V group predominated at first, then the V group disappeared by Day 6, and the Ps and the Ac groups became dominant (Fig. 6).

In both experiments using phyto- and zooplankton, the bacteria of the Ps group at first became dominant during the earlier stages of decomposition and again after a long incubation period (Fig. 5 and 6). However, the latter Ps group, present after long incubation, showed a very slow growth rate, and therefore, seemed to be different from the Ps group that predominated in the early stages of decomposition (see 'Discussion').

Assimilation activities of attached and free-living bacteria are summarized in Tables 1 and 2. In general, V_{max} values decreased as decomposition proceeded in

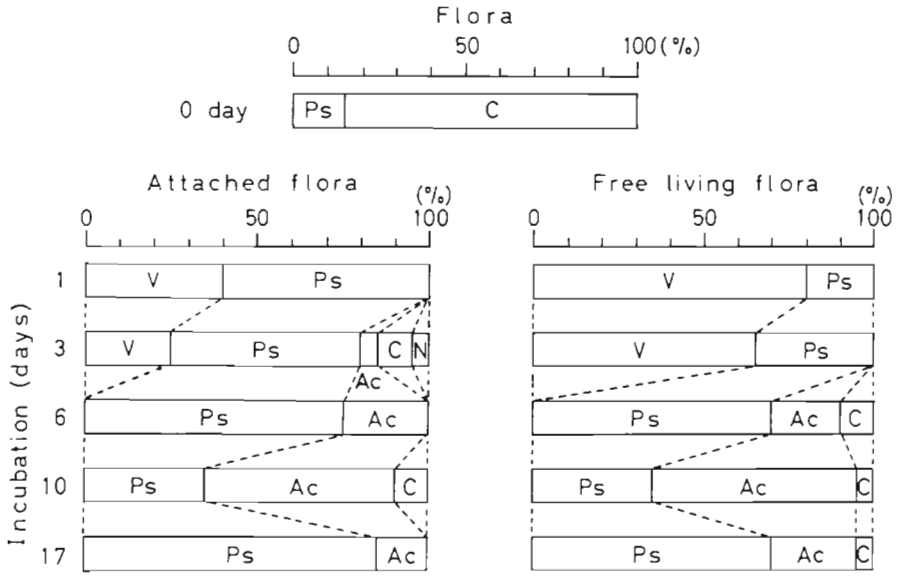

Fig. 6. Fluctuation of bacterial flora during decomposition of *Tigriopus japonicus*. See text for explanation of symbols

Table 1. Fluctuations in V_{max}, $K_t + S_n$, turn-over time (T_t), total cell count (TC) and specific activity (SA) of bacterial communities during decomposition of *Skeletonema costatum*

Days	V_{max} ($\mu g\ C\ l^{-1}\ h^{-1}$)		$K_t + S_n$ ($\mu g\ C\ l^{-1}$)		T_t (h)		TC ($\times 10^8$ cells l^{-1})		SA••• ($\times 10^{-9}$ $\mu g\ C\ cell^{-1}\ h^{-1}$)	
	A•	F••	A	F	A	F	A	F	A	F
1	0.127	2.21	16.1	8.84	126	3.99	0.168	13.0	7.58	1.70
4	0.122	0.732	7.50	20.0	61.6	27.3	0.659	8.44	1.85	0.867
21	0.084	0.130	5.89	4.17	70.0	32.2	0.695	5.73	1.21	0.226

• Attached bacteria
•• Free-living bacteria
••• Specific activity = V_{max}/TC

Table 2. Fluctuations in V_{max}, $K_t + S_n$, turn-over time (T_t), total cell count (TC) and specific activity (SA) of bacterial communities during decomposition of *Tigriopus japonicus*

Days	V_{max} ($\mu g\ C\ l^{-1}\ h^{-1}$)		$K_t + S_n$ ($\mu g\ C\ l^{-1}$)		T_t (h)		TC ($\times 10^9$ cells l^{-1})		SA••• ($\times 10^{-9}$ $\mu g\ C\ cell^{-1}\ h^{-1}$)	
	A•	F••	A	F	A	F	A	F	A	F
2	62.0	154	29.7	83.4	0.48	0.54	43.3	123	1.43	1.25
4	53.0	2.04	65.5	72.2	1.24	35.4	35.4	78.3	1.50	0.026
10	13.5	0.411	34.6	28.4	2.56	69.1	9.26	2.68	1.46	0.153
22	5.35	0.599	18.4	15.7	3.43	26.3	4.75	1.90	1.13	0.315
64	0.266	0.599	19.6	28.0	73.5	46.8	1.74	1.47	0.153	0.408

• Attached bacteria
•• Free-living bacteria
••• Specific Activity = V_{max}/TC

both phyto- and zooplankton experiments. However, the specific activity (V_{max}/total cell count) of attached bacteria was quite different from that of free-living bacteria. Attached bacteria maintained high specific activities until Day 20, while the specific activity of free-living bacteria showed high values only during the first few days and thereafter decreased rapidly. The specific activity of attached bacteria was often 10 times higher than that of free-living bacteria.

DISCUSSION

During decomposition of phytoplankton, fluctuations in the number of heterotrophic bacteria showed trends similar to those reported previously (Fukami et al., 1981). During the first few days, the number of free-living bacteria exceeded that of attached bacteria. Although the number of free-living bacteria decreased rapidly, the high count of attached bacteria was maintained for a longer period (Fig. 2 and 3). In a previous study (Fukami et al., 1981), using *Chlorella* sp., the number of attached bacteria was higher than that of free-living bacteria throughout the experiment. This discrepancy with the present results is due to the difference in pore size of the filter used to separate attached and free-living bacteria. In the present study, a 5.0 μm Nuclepore filter was used, whereas a 2.7 μm glassfiber filter (Whatman GF/D) was used by Fukami et al. (1981). Apparently, more free-living bacteria are retained on the 2.7 μm filter and as a result are counted as 'attached' bacteria.

The fact that a high count of attached bacteria was maintained for a long time suggests that attached bacteria utilized POM as a substrate and grew on it.

Fukami et al. (1981) indicated that the community of bacteria attached to POM was quite different from that of free-living bacteria both in terms of composition of the bacterial flora and in terms of their potential ability to decompose several organic substrates. In the present study, however, the differences between attached and free-living bacteria were not so clear (Fig. 5 and 6). The differences reported by Fukami et al. (1981), however, were obtained only from isolates which had grown on agar medium. The assimilation activity determined with total bacterial communities using labelled substrate (Tables 1 and 2) indicates that the specific activity of individual cells, that is the maximum assimilation velocity per individual cell (V_{max}/total cell count), shows significant differences between

the 2 bacterial communities. These results strongly suggest that attached bacteria are actively metabolizing, while the activity of free-living bacteria was quite low except immediately after the input of organic matter. Hodson et al. (1981) reported that per-cell uptake rate (specific activity) of attached bacteria was 1 or 2 orders of magnitude higher than that of free-living bacteria in natural seawater. Hodson et al. (1981) attributed the difference between the 2 communities to the difference in cell size; they reported that the activity per unit cell volume is about the same in both attached and free-living communities. In this study, cell volume or cell biomass was not determined but cell size probably did not differ greatly between the 2 communities. A previous report and the results of the present study support the speculation by Fukami et al. (1983b) that, when POM is supplied to an environment, heterotrophic bacteria will become associated with particulate matter and start to metabolize actively on POM. Azam and Hodson (1977) mentioned that the assimilation activity of the free-living community (passed through a 3 μm filter) was much higher than that of the attached community. In their investigation, however, they stated that the number of free-living bacteria was much higher than that of attached bacteria and their paper did not state the per-cell specific activity.

Although bacteria of the V group hardly appeared during phytoplankton decomposition (Fukami et al., 1981; Fig. 5 of this paper), it predominated during zooplankton decomposition (Fig. 6). Simidu et al. (1971) investigated the generic composition of bacteria associated with zooplankton and reported that more than two-thirds of the isolates were *Vibrio* spp. While growth of marine *Vibrio* spp. were stimulated by zooplankton (Kogure et al., 1980), it was inhibited easily by phytoplankton metabolite (Simidu et al., 1977; Kogure et al., 1979). From these reports and the present study it is suggested that bacteria of the V group, which may consist almost exclusively of Vibrionaceae, are not the main decomposers of phytoplankton but are involved in the decomposition of zooplankton. The abundance of V group bacteria during the early stages of zooplankton decomposition suggests that the proteolytic and the chitinolytic ability of V group bacteria is of significance in the degradation of zooplankton.

After addition of fresh plankton material, the Ps group again dominated initially, and then similar changes could be traced in the bacterial community (Fig. 5). This shows that the fluctuation in the bacterial community is a 'one-way' regular change and that there is a succession in the bacterial community involved in POM decomposition. During phytoplankton decomposition, the following successional changes in the flora were observed: Ps → Ac → C → Ps*. During zooplankton decomposition, the succession was: V → Ps → Ac → C → Ps*. Ps* which appeared in the last stage of the succession had a very low growth rate and it is clearly distinguished from the Ps group which predominated in the early stages of decomposition. In field observations, bacteria of the Ps* group with low growth rates were found abundantly in deeper layers (ca. 800 m) of the water column (Fukami, 1982). From these results, it is suspected that the bacteria of this group may utilize residual organic matter and may be the final decomposers of POM. However, further pertinent studies must be performed.

In addition to changes in floral composition, the high specific activity of bacteria attached to 'fresh' POM gradually decreased, and on Day 64 of zooplankton decomposition, by which time most of the POM would be rather 'old', the activity became as low as that of free-living bacteria (Table 2). This implies that the flora and the biochemical activity of attached bacteria depend on 'age' or chemical composition of the particulate materials which bacteria are associated with. Conversely, we may guess the origin and age of POM, i.e. whether it is fresh or old, by determining the composition or the activity or the attached bacterial community.

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LITERATURE CITED

Azam, F., Hodson, R. E. (1977). Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. 22: 492–501

Azam, F., Holm-Hansen, O. (1973). Use of tritiated substrates in the study of heterotrophy in seawater. Mar. Biol. 23: 191–196

Ferguson, R. L., Rublee, P. (1976). Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. 21: 141–145

Fukami, K. (1982). Microbiological study on the decomposition process of particulate organic matter in marine ecosystem. Ph. D. thesis, University of Tokyo

Fukami, K., Simidu, U., Taga, N. (1981). Fluctuation of the communities of heterotrophic bacteria during the decomposition process of phytoplankton. J. exp. mar. Biol. Ecol. 55: 171–184

Fukami, K., Simidu, U., Taga, N. (1983a). Change in a bacterial population during the process of degradation of a phytoplankton bloom in a brackish lake. Mar Biol. 76: 253–255

Fukami, K., Simidu, U., Taga, N. (1983b). Distribution of heterotrophic bacteria in relation to the concentration of particulate organic matter in seawater. Can. J. Microbiol. 29: 570–575

Fukami, K., Simidu, U., Taga, N. (1985). Microbial decomposition of phyto- and zooplankton in seawater I. Changes in organic matter Mar. Ecol. Prog. Ser 21: 1–5

Goulder, R. (1976). Relationships between suspended solids

and standing crops and activities of bacteria in an estuary during a neap-spring-neap tidal cycle. Oecologia (Berl.) 24: 83–90

Goulder, R. (1977). Attached and free bacteria in an estuary with abundant suspended solids. J. appl. Bacteriol. 43: 399–405

Griffiths, R. P., Hanus, F. J., Morita, R. Y. (1974). The effects of various water-sample treatments on the apparent uptake of glutamic acid by natural marine microbial populations. Can. J. Microbiol. 20: 1261–1266

Hodson, R. E., Maccubbin, A. E., Pomeroy, L. R. (1981). Dissolved adenosine triphosphate utilization by free-living and attached bacterioplankton. Mar. Biol. 64: 43–51

Kogure, K., Simidu, U., Taga, N. (1979). Effect of *Skeletonema costatum* (Grev.) Cleve on the growth of marine bacteria. J. exp. mar. Biol. Ecol. 36: 201–216

Kogure, K., Simidu, U., Taga, N. (1980). Effect of phyto- and zooplankton on the growth of marine bacteria in filtered seawater. Bull. Japan. Soc. scient. Fish. 46: 323–326

Oppenheimer, C. H., ZoBell, C. E. (1952). The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. J. mar. Res. 11: 10–18

Paerl, H. W. (1973). Detritus in Lake Tahoe: structural modification by attached microflora. Science, N.Y. 180: 496–498

Paerl, H. W., Merkel, S. M. (1982). Differential phosphorus assimilation in attached vs. unattached microorganisms. Arch. Hydrobiol. 93: 125–134

Simidu, U., Ashino, K., Kaneko, E. (1971). Bacterial flora of phyto- and zooplankton in the inshore water of Japan. Can. J. Microbiol. 17: 1157–1160

Simidu, U., Kaneko, E., Taga, N. (1977). Microbiological studies of Tokyo Bay. Microbiol. Ecol. 3: 173–191

Wiebe, W. J., Pomeroy, L. R. (1972). Microorganisms and their association with aggregates and detritus in the sea: a microscopic study. Memorie Ist. ital. Idrobiol. 29 (Suppl.): 325–352

Wilson, C. A., Stevenson, L. H. (1980). The dynamics of the bacterial population associated with a salt marsh. J. exp. mar. Biol. Ecol. 48: 123–138

Wright, R. T., Hobbie, J. E. (1966). Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47: 447–464

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