Comparison of DNA and protein synthesis rates of bacterial assemblages between coral reef waters and pelagic waters in tropical ocean

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ABSTRACT: DNA and protein synthesis activities of Pacific bacterial assemblages in coral reefs of Ponape Island (eutrophic water), Majero Atoll (mesotrophic water) and pelagic waters (oligotrophic water) were estimated by measuring incorporation rates of ^{3}H-thymidine and ^{3}H-leucine into the TCA-insoluble fraction (TdR_{DNA}, Leu_{prot}). Bacterial production rates estimated by TdR_{DNA} were 0.14 to 0.94 (average 0.52) $\times 10^4$ cells ml^{-1} h^{-1} in the pelagic water and 0.93 to 26 (average 10) $\times 10^4$ cells ml^{-1} h^{-1} in the eutrophic water Obligate oligotrophs predominated in the pelagic water, but facultative oligotrophs were dominant in the eutrophic water. In the eutrophic water protein synthesis rates expressed by Leu_{prot} were positively correlated to DNA synthesis rates expressed by TdR_{DNA}; however, in the oligotrophic pelagic water, there was no such correlation. Uptake activities of leucine at low concentration were maintained at relatively high levels in the oligotrophic water although ^{3}H-thymidine incorporation rates were extremely low. From these results, it is suggested that bacterial assemblages in oligotrophic waters have an uptake system with high affinity to substrates and utilize metabolic energy to achieve substrate uptake and protein synthesis preferentially to DNA synthesis and reproduction, and that these properties were mostly due to obligately oligotrophic bacteria adapted to a low nutrient environment.

INTRODUCTION

Many reports have shown that most marine pelagic bacteria adapt to oligotrophic environments and prefer lower nutrient conditions (less than 10 mgC l^{-1}) to higher nutrient conditions (Carlucci & Shimp 1976, Akagi et al. 1977, Ishida et al. 1986). Oligotrophic bacteria can tentatively be divided into 2 groups: obligate oligotrophs and facultative oligotrophs (Ishida et al. 1980, 1986). The latter authors define obligate oligotrophs as bacteria that can grow in a low nutrient medium (0.5 to 5 mgC l^{-1}) but not in a high nutrient medium, such as ZoBell 2216E. Facultative oligotrophs can grow in a relatively wide range of nutrient concentration. Previous results, using the ^{14}C-MPN method (Ishida et al. 1986, Eguchi & Ishida 1990), indicated that obligate oligotrophs were predominant in pelagic oceans with extremely low nutrient concentrations. On the other hand, in eutrophic coastal waters facultative oligotrophs and/or eutrophs which can grow in a high nutrient medium were dominant. These results led us to believe that heterotrophic bacterial assemblages in

oligotrophic pelagic waters must possess different physiological properties from those of bacteria in eutrophic waters, with regard to the capability of effective nutrient uptake at low concentrations, and low maintenance energy and growth.

Recently, many studies on bacterial production have revealed that a large soluble fraction derived from primary production is utilized by heterotrophic bacteria in marine ecosystems and converted into bacterial biomass (Ducklow & Kirchman 1983, Ducklow et al. 1986, Cole et al. 1988, Hagström et al. 1988, Rosenberg et al. 1990). Bacterial production rates were generally estimated by using a ^{3}H-thymidine method (Fuhrman & Azam 1982). Azam & Fuhrman (1984) summarized that thymidine incorporation has already yielded useful estimates of bacterial production in seawater. Several problems, however, have arisen. Establishing conversion factors from thymidine incorporation rates to bacterial production rates is one of the most serious. Moreover it is possible that the conversion factor could be variable depending on bacterial physiological conditions as well as community structure.

Kirchman et al. (1985) proposed that the rate of protein synthesis estimated from radiolabeled leucine incorporation into the hot trichloroacetic acid (hot-TCA) insoluble fraction may be a more direct measure of heterotrophic activity in aquatic environments (Chin-Leo & Kirchman 1988, Kirchman & Hoch 1988, Kirchman et al. 1989). A simultaneous application of leucine and thymidine methods is particularly useful in elucidating the physiological dynamics of bacterial assemblages in marine environments.

In this paper, we report on determinations of bacterial production rates in eutrophic waters over tropical coral reefs and in oligotrophic waters in the pelagic ocean, and attempt to clarify the physiological differences in DNA and protein synthesis activities between oligotrophic and eutrophic bacterial assemblages. Subsequently, we discuss the physiological properties of oligotrophic bacterial assemblages: how these assemblages utilize a limited nutrient and energy source to maintain their heterotrophic activities.

MATERIALS AND METHODS

Sampling sites. Water samples were collected during the KH-88-1 cruise of RV 'Hakuho-maru' (Ocean Research Institute, University of Tokyo) in March 1988. The sampling area in the W Pacific Ocean is illustrated in Fig. 1A, B, C. At Majero Atoll, 3 water samples were taken at Stn MA, which was situated just on the coral reef, 2 (MA1 and MA3) during ebb tide and 1 (MA2) during flood tide. Stations at Ponape Island were on the reef fringe. Water samples at Stns PA through PG were collected during ebb tide on 14 March (PA1) and 15 March (all other stations).

Surface waters were collected using sterile glass bottles which were precombusted at 450 °C for 1.5 h for removal of any organic contamination. At Stn 28, which was situated in a pelagic area with a water depth of 1860 m, water samples were collected from 0, 10, 20, 30 and 50 m depths. Deeper water samples were taken with a rosette multisampler. These water samples were stored at 5 °C until they were used in the experiments.

DOC determination. Each water sample was filtered with a glass fiber filter (Whatman, GF/F) combusted at 450 °C for 1.5 h. Dissolved organic carbon (DOC) concentrations in filtrates were measured with a TOC meter (Shimadzu, TOC-500) after pretreatment with concentrated HCl.

Incorporation rates of ^{3}H-thymidine and ^{3}H-leucine. We investigated incorporation rates of ^{3}H-thymidine into the cold-TCA insoluble fraction (TdR_{DNA}) and of ^{3}H-leucine into the hot-TCA insoluble fraction (Leu_{prot}) for estimation of DNA and protein synthesis rates. Triplicate water samples of 20 ml for pelagic waters
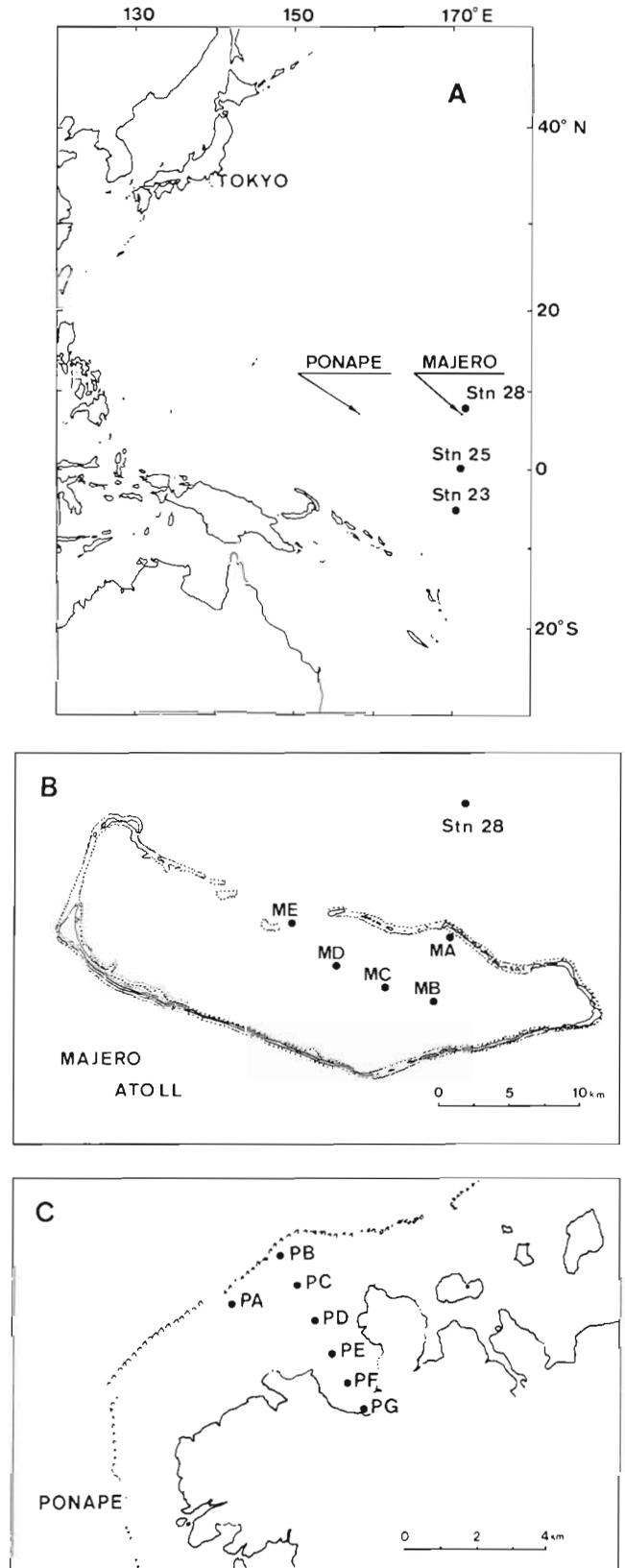

Fig. 1. Location of sampling stations in (A) the Pacific Ocean, (B) Majero Atoll and (C) Ponape Island

and 10 ml for near-shore water were incubated with either 5 nM methyl-^{3}H-thymidine (56 Ci mmol^{-1}, CEA) or 5 nM L-^{3}H-leucine (120 Ci mmol^{-1}, CEA) at in situ temperature. After 1 h incubation, the reaction was stopped by adding ice-cold TCA (5 % of final concentration). Samples for TdR$_{DNA}$ were cooled on ice for 5 min to extract a macromolecular fraction. Samples for Leu$_{prot}$ were heated for 1 h at 80 °C to hydrolyze all macromolecules except proteins. The insoluble fraction of each sample was collected onto a membrane filter (pore size, 0.2 μm), rinsed with 10 ml of 5 % ice-cold TCA twice, and then transferred into a glass vial containing 5 ml of scintillation cocktail (Aquasol 2). Incorporated radioactivities were measured with a liquid scintillation counter (Aloka LSC502). In order to estimate a bacterial production rate, ^{3}H-thymidine incorporation rates (TdR$_{DNA}$) were multiplied by 1.7×10^{18} cells mol^{-1} thymidine (Fuhrman & Azam 1982).

To examine the total assimilation rate of leucine into whole cell materials, Leu$_{ass}$, water samples were incubated with 5 nM ^{3}H-leucine in the same manner as described above. After incubation, bacterial cells were collected on membrane filters as quickly as possible without TCA treatment, rinsed with cold filtered seawater twice, and then their radioactivities were determined. Radioactivities of formalin-killed water samples (2 % final concentration) were measured for abiotic adsorption of radioactivity.

Bacterial counting. Total bacterial abundance was measured with epifluorescence microscopy after DAPI staining (Porter & Feig 1980). The number of viable oligotrophic bacteria was counted by the modified Ishida's MPN method (Ishida & Kadota 1979, Ishida et al. 1980). Water samples were diluted appropriately and samples of each dilution step were inoculated into 5 replicate test tubes containing 3 ml of ST10^{-4} medium, which included 0.5 mg trypticase peptone (BBL) and 0.05 mg yeast extract (Difco) in 1 l of aged seawater (ASW). After 1 mo incubation at 25 °C, we detected bacterial growth in each tube by direct observation under an epifluorescence microscope, and the most probable number (MPN) was determined from a series of numbers for bacterial growth-positive tubes. These bacterial numbers are regarded as values for total oligotrophic bacteria (TO), which are able to grow in a low nutrient medium like ST10^{-4} medium. In addition, a part of a culture in each tube was inoculated secondarily into freshly prepared ST10^{-1} medium, which contained 1000-fold higher nutrient concentrations than ST10^{-4}. After 2 wk incubation, bacterial growth was detected by turbidity in each tube and an MPN value again obtained. The MPN values from the secondary inoculum were regarded as those of facultative oligotrophs (FO), which could grow in both low (ST10^{-4}) and high (ST10^{-1}) nutrient media.

RESULTS

Numbers of bacteria of several categories, DOC and chlorophyll a concentrations at each sampling point are presented in Table 1. DOC concentrations in surface waters of the inlet of Majero Atoll (Stns MD and ME) and those in pelagic waters were around 1 mgC l^{-1} or less. However, DOC concentrations in the inner atoll (Stns MB and MC) and on the coral reef (Stn MA) at Majero, and at all sampling points at Ponape, were generally more than 2 mgC l^{-1}. DOC concentrations, chlorophyll a concentrations and total bacterial numbers (DAPI-DC) in coral reef waters of Ponape and Majero were higher than those at the inlet of Majero Atoll and in pelagic waters (Table 1). Although the chlorophyll a concentration was notably low at Stn PG, the innermost point at Ponape, the DOC concentration was high (4.65 mgC l^{-1}), resulting from the location of station just beside the shore where many pollutants were present.

MPN counts of total oligotrophs (TO) in pelagic waters were generally lower (0.3 to 2.3×10^3 cells ml^{-1}) than those at Majero and Ponape (Table 1). At Majero and Ponape, on the other hand, facultative oligotrophs (FO), which could grow in both high and low nutrient media, often predominated. In pelagic waters, the percentages of obligate oligotrophs to total oligotrophs, calculated as $(1 - FO/TO) \times 100$, were usually more than 80 % with one exception (10 m depth of Stn 28; Table 1).

A vertical profile of bacterial production rates at Stn 28 and horizontal profiles of those in Majero and Ponape derived from ^{3}H-thymidine incorporation rates into cold-TCA insoluble fraction (TdR$_{DNA}$) are presented in Figs. 2 & 3A, B. Bacterial production rates in pelagic waters and at the inlet of Majero Atoll were estimated to be at most 10^4 cells ml^{-1} h^{-1}. The horizontal profile of bacterial production at Ponape (Fig. 3B) shows variations from 9.3×10^3 cells ml^{-1} h^{-1} at Stn PA1 to 2.6×10^5 cells ml^{-1} h^{-1} at Stn PG. Bacterial production rates showed a statistically significant positive correlation to DOC concentrations ($r^2 = 0.67$, $p < 0.01$).

The correlation between TdR$_{DNA}$ and leucine incorporation rates into the hot-TCA insoluble fraction (Leu$_{prot}$) is shown in Fig. 4. TdR$_{DNA}$ of bacterial assemblages in eutrophic water, such as Ponape water, was positively correlated to Leu$_{prot}$. When values of TdR$_{DNA}$ were lower than 10^{-14} mol ml^{-1} h^{-1} in Majero and pelagic waters, values of Leu$_{prot}$ remained nearly constant, at around a few 10^{-14} mol ml^{-1} h^{-1}, although TdR$_{DNA}$ fluctuated significantly.

Total amounts of leucine assimilated into whole cell materials (Leu$_{ass}$) (see 'Materials and methods') were measured at Stn 28 and in Majero waters. In Fig. 5A TdR$_{DNA}$ is plotted against Leu$_{ass}$ and in Fig. 5B against

Table 1. Bacterial profiles, chlorophyll a concentrations and DOC concentrations in pelagic water, Majero Atoll and Ponape water. TO: number of total oligotrophs counted by MPN method with ST10^{-4} medium; FO: number of facultative oligotrophs; OO/FO: percentage of obligate oligotrophs (OO), $(1 - FO/TO) \times 100$; DAPI-DC: total bacterial number counted by direct counting method with DAPI stain; DOC: dissolved organic carbon concentration; chl a: chlorophyll a concentration from 'Preliminary Report of The Hakuho Maru Cruise KH-88-1'. ND: no data

Station	Depth (m)	TO (cells ml^{-1})	FO (cells ml^{-1})	OO/TO (%)	DAPI-DC (cell ml^{-1})	DOC (µgC l^{-1})	Chl a (µg l^{-1})
Pelagic water							
Stn 28	0	2.3×10^3	2.3×10^2	90.0	8.3×10^5	0.86	0.06
	10	1.7×10^3	1.3×10^3	23.5	1.3×10^6	1.71	0.06
	20	3.3×10^2	2.3×10	93.0	8.6×10^5	1.34	0.05
	30	1.3×10^3	2.3×10^2	82.3	6.2×10^5	1.36	0.06
	50	4.6×10^2	2.3×10	95.0	8.3×10^5	1.24	0.06
Stn 23	0	1.3×10^3	1.7×10^2	86.9	8.4×10^5	1.02	ND
Stn 25	0	7.9×10^2	1.3×10^2	83.5	8.1×10^5	0.96	ND
Majero							
MA1	0	4.9×10^3	2.3×10^3	53.1	9.5×10^5	2.16	ND
MA2	0	1.7×10^4	1.3×10^4	23.5	1.4×10^6	2.25	ND
MA3	0	2.3×10^3	2.3×10^3	0.0	9.7×10^5	2.15	ND
MB	0	4.9×10^3	4.9×10^3	0.0	1.2×10^6	2.09	ND
MC	0	1.7×10^4	7.0×10^3	58.8	1.1×10^6	2.08	ND
MD	0	2.3×10^3	7.9×10^2	65.7	8.0×10^5	0.82	ND
ME	0	7.9×10^3	1.3×10^3	83.5	1.0×10^6	0.64	ND
Ponape							
PA1	0	1.2×10^4	2.2×10^3	81.7	1.1×10^6	1.66	ND
PA2	0	7.9×10^3	3.3×10^3	58.2	7.8×10^5	1.95	ND
PB	0	2.3×10^3	1.7×10^3	26.1	8.8×10^5	2.74	0.10
PC	0	7.0×10^2	4.6×10^2	34.3	1.0×10^6	1.95	0.32
PD	0	4.9×10^3	4.9×10^3	0.0	1.6×10^6	2.17	0.39
PE	0	2.2×10^3	1.7×10^3	22.7	1.1×10^6	2.04	0.27
PF	0	4.6×10^3	1.8×10^3	60.9	1.3×10^6	4.26	0.57
PG	0	1.4×10^4	1.1×10^3	92.1	1.1×10^6	4.65	0.06

percentage of Leu$_{prot}$ to Leu$_{ass}$. Leu$_{ass}$ of the bacterial assemblages at 3 depths (20, 30 and 50 m) at Stn 28 and at 1 station at Majero (Stn ME) were significantly higher than those in more eutrophic waters (Stns MA, MB, MC and MD), although DOC concentrations were low, and consequently, bacterial production rates estimated by TdR$_{DNA}$ were low (Fig. 3A). The latter resulted in only 42.5 to 68.6 % of leucine taken up into bacterial cells being incorporated into the protein fraction in these areas, while bacterial assemblages of other stations and depths incorporated nearly all the leucine into the protein fraction. Leucine uptake activities of the 3 surface water samples in the pelagic area were also high, and percentages of Leu$_{prot}$ to Leu$_{ass}$ were between 34.3 and 59.4 % (mean value 50.7 %) (data not shown).

DISCUSSION

As bacterial assemblages in the pelagic ocean are usually exposed to extremely low nutrient environments, some scientists have believed that most bacteria must be gradually starving to death or have already died (Morita 1985). However, using the micro-autoradiographic method, Douglas et al. (1987) showed that a large part of the total bacteria in natural environments assimilated radiolabeled glutamate (> 50 %)
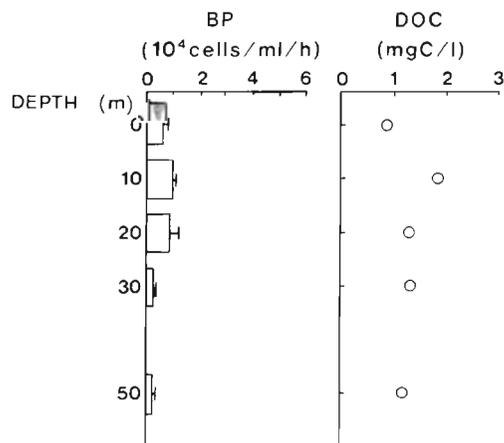

Fig. 2. Vertical profiles of bacterial productions (BP) and dissolved organic carbon (DOC) concentrations at Stn 28
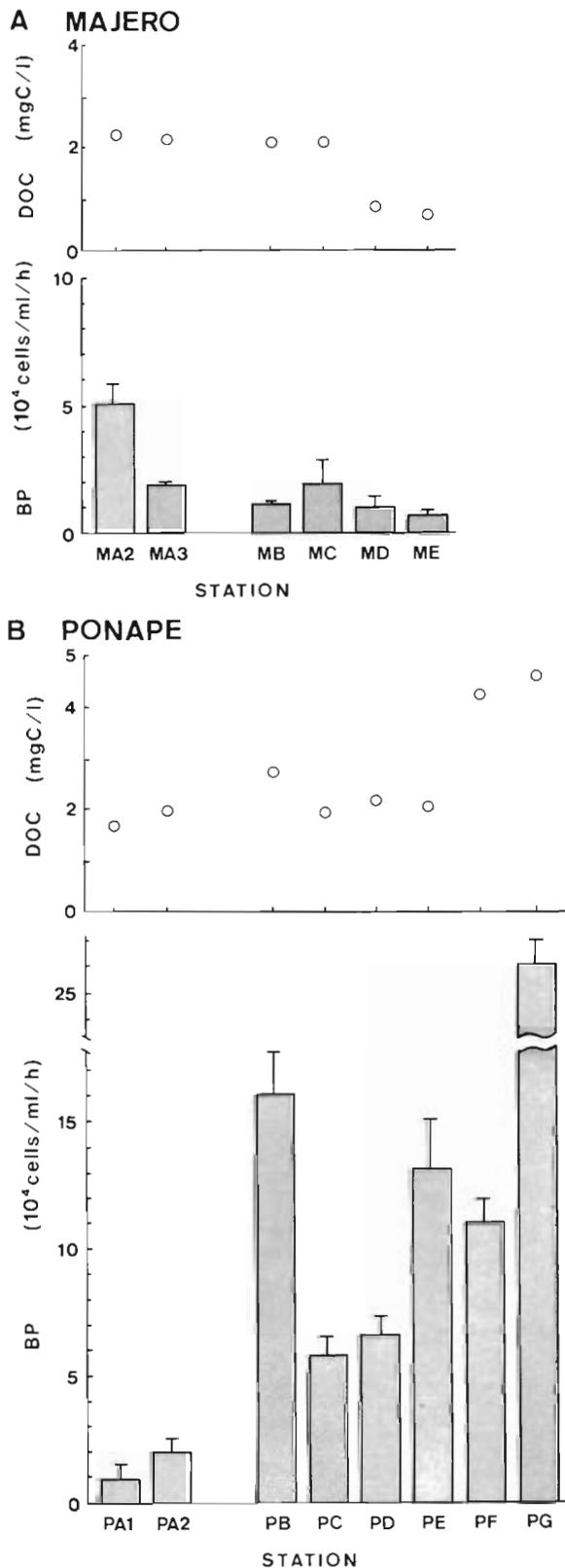
A MAJERO

B PONAPE

Fig. 3. Horizontal profiles of bacterial production (BP) and DOC concentrations at (A) Majero and (B) Ponape. MA2 and MA3 samples were collected at 11:40 h (ebb) and 15:00 h (flood) respectively on 5 March 1988 at Stn MA. PA1 and PA2 samples were collected on 14 and 15 March respectively at Stn PA
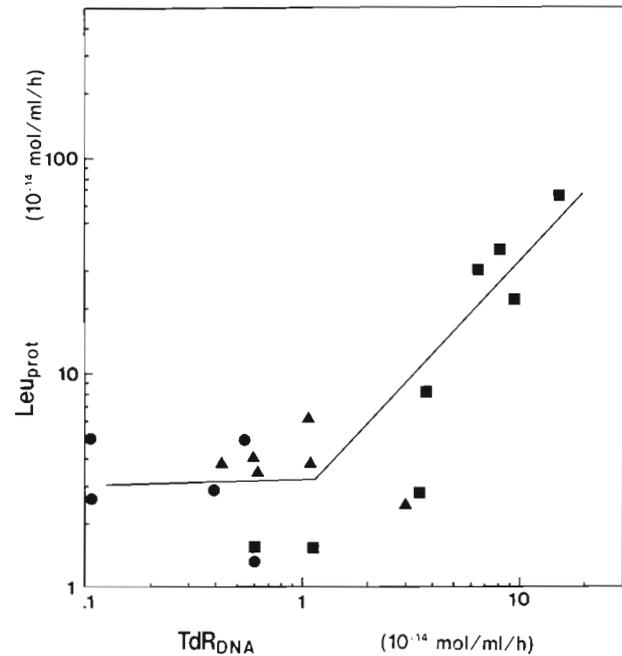

Fig. 4. Overall relationship between ^{3}H-thymidine incorporation rates (TdR$_{DNA}$) and ^{3}H-leucine incorporation rates (Leu$_{Prot}$). Pelagic water samples (●), samples from Majero (▲), and samples from Ponape (■) were plotted

and thymidine (> 10 %). A recent study using the thymidine method also revealed that bacterial production in oligotrophic Mediterranean Sea water required 60 % of primary production (Hagström et al. 1988). These results support the recognition that most bacteria are not dead or dying, but metabolically active, even in oligotrophic environments.

In the present study, average DOC concentrations and total bacterial numbers (DAPI-DC) in pelagic waters were 1.07 µgC 1^{-1} and 8.7×10^5 cells ml^{-1}, respectively, and were generally lower than those in the coral reef waters of Majero and Ponape where average DOC and DAPI-DC were high (Table 1). Viable bacterial abundances counted by the MPN method with a low nutrient medium (total oligotrophic bacteria, TO) were higher than those counted with high nutrient agar plates, and most bacteria (> 82.3 %) in the pelagic waters, except for those in water from 10 m depth at Stn 28, were obligate oligotrophs (OO) (Table 1). This result confirms a previous proposition (Ishida et al. 1986) that obligate oligotrophs were dominant in oligotrophic pelagic oceans and played the most significant role among microbial communities. In the coral reef waters of Majero and Ponape, on the other hand, since DOC concentrations were higher, TO were more abundant than in pelagic water with higher percentages of facultative oligotrophs (FO) (Table 1). Coral reef waters in Ponape and Majero seemed to be eutrophic areas.

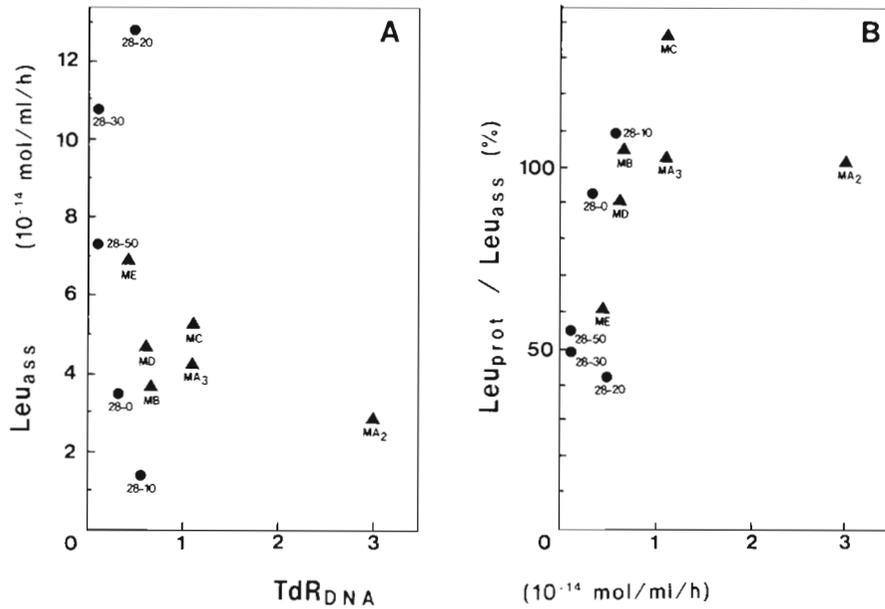Bacterial production rates of pelagic water ranged

Fig. 5. Relationship between TdR_{DNA} and (A) assimilation rates of ^3H-leucine into bacterial cells (Leu_{ass}) and (B) ratios of Leu_{Prot} to Leu_{ass}

from 0.14 to 0.94 (average 0.52) $\times$ 10^4 cells ml^{-1} h^{-1} and those of fringing reef waters in Ponape were 0.93 to 26 (average 10) $\times$ 10^4 cells ml^{-1} h^{-1}, i.e. 1 or 2 orders of magnitude higher (Figs. 2 & 3B). From the inner area (Stns MA, MB and MC) to the inlet (Stns MD and ME) of Majero Atoll, bacterial production activities decreased along with the decrease of DOC concentrations (Fig. 3). In the inlet area, DOC concentrations and bacterial production activities were nearly equal to those in the pelagic waters. On the other hand, bacterial production and DAPI-DC at Stn MA during ebb tide (MA2) were as high as in fringing reef water at Ponape, while those during flood tide (MA1 and MA3) were lower. These results can be explained by the fact that pelagic water could easily flow into the inner part with tidal current, as the inner area was wide and deep (ca 30 m depth), and inlet water of the atoll would be diluted by pelagic waters. At Ponape, however, the fringing reef around the island extends a few kilometers from the island. Therefore, flow of pelagic water over the reef to dilute inner reef water may be limited. Moreover, much run off water could be supplied from the island, resulting in water inside the fringing reef being more eutrophic than in Majero Atoll.

Many previous reports suggested that seasonal and regional fluctuations of bacterial production in the photic zone were controlled by the primary production (Cole et al. 1988), or by the concentration of dissolved organic matter (Kirchman et al. 1986). In this investigation, DOC concentrations and DNA synthesis rates (TdR_{DNA}) showed a statistically significant positive correlation ($r^2 = 0.67$, $p < 0.01$) (Figs. 2 & 3). In the pelagic water, however, protein synthesis activities (Leu_{prot}) were maintained at 3 to 5 $\times$ 10^{-14} mol ml^{-1} h^{-1}, even

when DOC concentration and TdR_{DNA} decreasd (Fig. 4). There was, in other words, no synchronization between protein and DNA syntheses by bacterial assemblages in low nutrient environments. To this phenomenon, several possible explanations can be offered: (1) oligotrophic bacteria could alter their growth rate on the sudden nutrient inflow and transiently enter an unbalanced growth stage; (2) bacteria incapable of growth, but capable of active metabolism of substrates are dominant in pelagic water; (3) oligotrophic bacteria in pelagic water could not incorporate extracellular thymidine for DNA synthesis; or (4) bacteria in pelagic water are different from those in eutrophic areas in regard to their macromolecular biosynthesis or uptake system.

(1) Bacteria are usually considered to be in a balanced growth stage, and then rates of all macromolecular syntheses are proportional to specific growth rates. The moment bacteria encounter new and fresh environments and their growth rates shift (up) to a new stage, synchronization of their macromolecular synthesis is disturbed and they transiently enter unbalanced growing stage (Kirchman et al. 1986). However, in the present study, 5 nM of ^3H-leucine was added to a final concentration. This leucine concentration would not be high enough to stimulate bacterial growth, because it is almost equivalent to the in situ leucine concentration in pelagic oceans (Lee & Bada 1977, Eguchi & Ishida 1990).

(2) There are few studies on the abundance of bacteria which actually take up substrates in natural environments. Results from the autoradiographic technique in eutrophic areas (Fuhrman & Azam 1982, Tabor & Neihof 1982) suggested that the number of bacteria which could incorporate thymidine was com-

parable to those taking up amino acids. In contrast, results on offshore waters of Nova Scotia (Douglas et al. 1987) indicated that the numbers of glutamate-incorporating bacteria were greater than those of thymidine-incorporating. These results suggested that the percentage of bacterial assemblage which did not incorporate thymidine, but actively metabolized amino acids, might be higher in the oligotrophic area. Douglas et al. proposed that some bacterial assemblages, living in pelagic ocean of low nutrient for a long time, may be forced to stop reproducing but still retained the ability to metabolize substrates actively. Many starvation experiments using culturable bacteria showed that a considerable proportion of bacteria still maintained respiratory activity, although viable cells decreased significantly after long-term starvation (Amy et al. 1983, Kurath & Morita 1983). Although growth rates of bacterial assemblages in natural environments are undoubtedly slow, most bacteria cannot be in a dormant stage because of their high secondary production activities (Azam & Cho 1987).

(3) There is another possibility that most growing bacteria in oligotrophic environments do not take up extracellular thymidine and do not assimilate it into the DNA fraction. Several previous reports pointed out that some bacterial cultures did not utilize extracellular thymidine for DNA synthesis (Ramsey 1974, Johnstone & Jones 1989, Jeffrey & Paul 1990). Novitsky (1983) reported in his work using microautoradiography that there were some dividing cells which did not incorporate ^{3}H-thymidine in a natural aquatic ecosystem.

(4) In their review, Hirsh et al. (1979) proposed physiological properties of an 'ideal' oligotroph adapting to a low nutrient flux environment. These were: (1) the ideal oligotroph must have substrate uptake mechanisms with high affinity in order to react to low nutrient inflow sensitively and to take it up quickly (Ishida et al. 1982); and (2) the ideal oligotroph spends valuable metabolic energy obtained from limited nutrients for the maintenance of a high affinity uptake system rather than for multiplication (DNA synthesis). In the present study, bacterial assemblages in oligotrophic areas could take up leucine of low concentration effectively even when DNA synthesis was suppressed (Fig. 5A). As the leucine assimilation rates of these bacterial assemblages were larger than the leucine incorporation rates into the protein fraction, about half of the leucine taken up into the cell was promptly utilized for incorporation into the protein fraction (Fig. 5B). This result suggests that bacteria in oligotrophic waters would consume most energy to maintain their efficient uptake systems.

Previous studies reported that nutrient deprivation caused some physiological changes of marine bacterial cells in cell volume, endogenous metabolism protein composition and so on (Novitsky & Morita 1978,

Geesey & Morita 1981, Kurath & Morita 1983, Mårdén et al. 1985, Nyström et al. 1988). A copiotrophic bacterium, S14, which possessed 2 leucine uptake systems of low and high affinities, acitvated the high-affinity system under starved conditions (Mårdén et al. 1987).

In our laboratory studies with strains of obligate oligotrophs and facultative oligotrophs isolated from pelagic water in the Sea of Japan, oligotrophs growing in a low nutrient medium (0.5 mg peptone l^{-1}) maintained relatively high leucine and protein synthesis activity, although DNA synthesis activity was lower, compared with those growing in a high nutrient medium (0.5 g peptone l^{-1}) (unpubl.). This result suggests that bacterial assemblages in oligotrophic environments mostly consume their energy for the maintenance of uptake system and metabolic activities. It was reported that *Escherichia coli* cells growing in a carbon-limited chemostat with a low growth rate maintained higher activities of potential protein synthesis than actual synthesis rate and this 'extra' protein synthesis activity had a great selective advantage in fluctuating environments (Koch 1971). These physiological properties of bacterial assemblages in oligotrophic areas may relate to the finding that small bacteria in such areas have a higher percentage of protein content to cell volume than large ones in eutrophic areas (Simon & Azam 1989).

The present study has clarified that metabolically active oligotrophic bacteria formed the majority of microbial communities in pelagic oceans, and suggested that these bacteria possess special physiological properties different from those of bacteria in eutrophic environments, due to genetical adaptation to a low nutrient flux environment.

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