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Abundance and Growth Characteristics of the Bacterioplankton inside and outside the Hydrothermal Vent Plumes in the North Fiji Basin*

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Abstract: Bacterioplankton were studied from the deep waters near the boundaries of hydrothermal vent plumes in the North Fiji Basin with special reference to their chemosynthetic sulfur-utilization. The bacterial abundance inside the plumes was greater than that outside the plumes. However,no clear difference in their growth rate was evident for samples collected inside and outside the plumes. The addition of thiosulfate had little influence on growth rate, because the population density of thiosulfate-utilizers near the boundary of hydrothermal plumes occupied only a small fraction of the bacterioplankton community.

1. Introduction

Active hydrothermalism in the rift of the North Fiji Basin has been intensively studied (KAIYO 87 Shipboard Party, 1988; HONZA *et al.*, 1989),and typical vent fauna composed of *Bathymodiolus, Alviniconcha, Munidopsis, Bythograea,* etc. were observed by the deep-towed TV camera (HASHIMOTO *et al.*, 1989), and the long-term observation system (MITSUZAWA *et al.*, 1989) both developed by Japan Marine Science and Technology Center. An active "white smoker" chimney and other vents in the area were carefully observed by the French submersible *Nautile* in 1989 (URABE *et al.*, 1990). Chemical studies show the vent plumes rising up to hundreds of metres and the occurrence of a "megaplume" in the North Fiji Basin (NOJIRI *et al.*, 1989). The occurrence of chemolithotrophic sulfur bacteria in the vent plumes was determined, with their active growth and CO_2 - uptake being certified experimentally (NAGANUMA *et al.*, 1989; SEKI and NAGANUMA , 1989; SEKI *et al.*, 1991).

This study aims at evaluating the abundance and growth characteristics of bacterioplankton communities inside and outside the boundary of vent plumes,with reference to their chemosynthetic sulfur-utilization. This research was part of "The Joint Research Program on the Rift System in the Pacific Ocean" funded by the Special Cooperation Fund of the Science and Technology Agency of Japan (STA) and the Institut Francais de Recherche pour l'Exploitation de la Mer of France (IFREMER) in cooperation with the Committee for the Coordination of Joint Prospecting for Mining Resources in South Pacific Offshore Areas (CCOP/SOPAC).

2. Materials and Methods

In November and December 1988, an integrated research program of geophysics, geology, geochemistry and biology was conducted along the rift axis during the KAIYO 88 cruise with R.V. *Kaiyo* (HONZA *et al.*, 1989). Bacteriological samples were collected at 12 sites along the rift axis by using Go-flo bottles and sterile Niskin bags (Go-flo samples and Niskin samples; SEKI *et al.*, 1991). The sample depths were from the bottom to more than 1000 m above the bottom. The sample locality, i.e. inside or outside plumes, could be precisely indicated by manganese concentration.

Immediately after collection, portions of the Go-flo samples for counting bacterial cells were fixed with 0.2 μm-filtered formalin at a final concentration of 2%. Bacterial cells in each 5-50 ml of the samples were counted by the combination fo membrane-filtration and epifluorescence microscopy (NAGANUMA *et al.*, 1989). Separate counts were made for: the

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total cells, growing cells (with diameter larger than 0.3 μm; MORITA, 1982), and dividing cells. The frequency of dividing cells (FDC; HAGSTROM *et al.*, 1979) was also estimated from the separate counts; this FDC value gives the *in situ* FDC.

The number of thiosulfate-utilizers was counted by the spread-plate method using 1.5%-agar plates (of TB medium; TUTTLE and JANNASCH, 1972). The agar plates were inoculated with the Niskin samples, and kept in a cold room (at about 4℃) in the dark for more than 4 weeks. The number of colonies counted represents in principle the abundance of thiosulfate-utilizers.

Growth rates of the bacterioplankton were determined immediately after the sampling, from the changing rate of bacterial cell numbers during the chemostat culture (JANNASCH, 1969). Vent plumes should be associated with chemosynthetic activity by sulfur oxidation; thus two media with reference to sulfur-utilization were used for the chemostat culture. The media for this purpose were: 1) thiosulfate-enriched TB medium, a liquid medium containing 1% of sodium thiosulfate in 75% aged seawater diluted with distilled water (TUTTLE and JANNASCH, 1972); and 2) thiosulfate-free TB medium. The chemostat cultures with two media were conducted in parallel at 1-2℃, in the dark at 1 atm and with a dilution rate of 0.2-0.3 hr^{-1}. The FDC, as well as growth, was determined during the first 12 hours of the chemostat culture. Thus the FDC-growth relationship in the chemostat culture was determined and converted to *in situ* FDC values which were used to calculate the in situ growth rate.

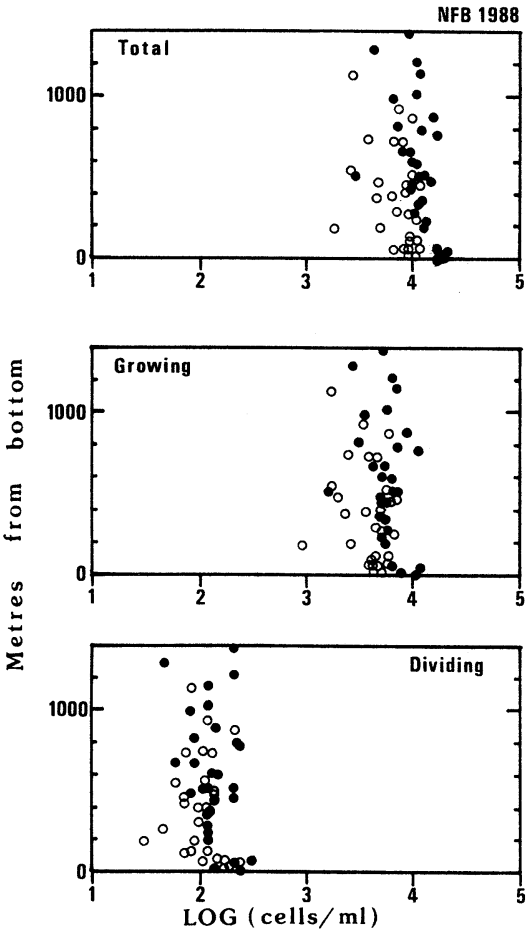

Fig. 1. Vertical distribution of total cells, growing cells and dividing cells of bacterioplankton in the bottom 1000 m layer of the North Fiji Basin. Closed and open circles indicate the bacterial abundance inside and outside the hydrothermal vent plumes, respectively.

3. Results and Discussion

3.1. Bacterial abundance

Total cell counts were within the range of $\times 10^3$-10^4 cells ml^{-1} throughout the water column in the bottom 1000 m, and no obvious relationships between depths and counts were shown (Fig. 1). Higher counts, however, were mostly found for the samples collected inside vent plumes, where the manganese concentrations were higher than the background concentration of 1-2 nM. Actually, the highest manganese concentrations of 5-16 nM were associated with the highest total bacterial counts of $1.0\text{-}2.1\times10^4$ cells ml^{-1}. A relationship between manganese concentration and total cell counts was evident for the bottom-most samples. and was expressed as follows (Fig. 2):

$$\log[\text{cells ml}^{-1}] = 3.74 + 0.54 \log[\text{manganese nM}]$$

with a statistical significance of $r^2=0.74$ ($n=10$, significant at 2.5% level). Hence, the bacterioplankton densities greater than $5.5\text{-}8.0\times10^3$ cells ml^{-1} had a linear relation with

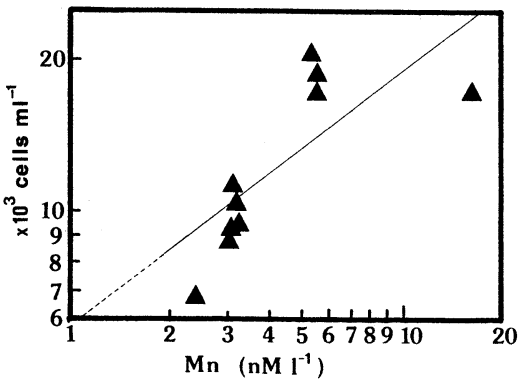

Fig. 2. Relationship between the manganese concentration and the total cells of bacterioplankton in the bottom-most water of the North Fiji Basin.

the manganese concentrations above 1-2 nM.

The counts of growing cells were almost within the range of $\times 10^3$ cells ml^{-1}, and the higher population densities were mostly observed inside the plumes (Fig. 1). The ratio of *total: growing* cell counts was 0.52 on an average. This means that about half of the total bacterioplankton were in the growing phase. No clear difference in the ratio was found among samples collected inside and outside the plumes.

The counts of dividing cells were within the range from $\times 10^1$ cells ml^{-1} to $\times 10^2$ cells ml^{-1}, and they were higher inside the plumes (Fig. 1). The *in situ* ratio of total: dividing cell count was 0.013 on an average, while the *in situ* ratio of growing: dividing cell counts was 0.024 on an average. No obvious difference in the FDC values was calculated between samples collected inside and outside the plumes.

There was an evident difference of bacterial abundance inside and outside vent plumes, as bacterioplankton occurred more abundantly inside the plumes. On the other hand, the ratio of total: growing cell counts and the *in situ* FDC estimations showed no difference for bacterioplankton inside and outside the plumes.

The fraction of thiosulfate-utilizers was found to be less than 1% of the total bacterioplankton. A previous study showed that a considerable fraction of the total counts was composed of thiosulfate-utilizers (NAGANUMA *et al.*, 1989). All the previous results were obtained from samples collected at plume centers where the manganese concentrations were higher. A lower abundance of thiosulfate-utilizers in this study indicates the samples were collected at locations closer to the edge or boundary of plumes.

3.2. Growth characteristics

Growth rates of the bacterioplankton, estimated from on-board cultivation immediately after sampling, ranged from nearly zero to 0.17 hr^{-1} (a generation time of 4.2 hrs). There was no clear difference between growth rates inside and outside vent plumes. The addition of thiosulfate had little influence on their growth, at least at the concentration used. Higher FDC values were, however, enumerated for the thiosulfate-enriched culture than in the non-enriched culture (Fig. 3), and the FDC-growth relationships were expressed as follows:

thiosulfate-enriched

$[FDC\ \%] = 0.89 + 4.56[\text{growth rate } hr^{-1}]$

$r^2 = 0.54$ (n=8, significant at 20% level)

non-enriched

$[FDC\ \%] = 0.61 + 4.45[\text{growth rate } hr^{-1}]$

$r^2 = 0.93$ (n=8, significant at 0.1% level)

total

$[FDC\ \%] = 0.70 + 5.26[\text{growth rate } hr^{-1}]$

$r^2 = 0.49$ (n=16, significant at 5% level)

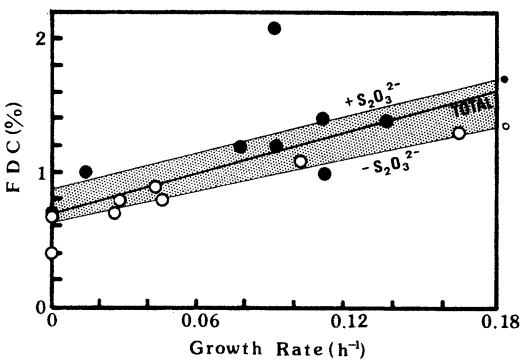

Fig. 3. Relationship between the growth rate and the frequency of dividing cells (FDC) measured by the chemostat culture. Closed and open circles indicate the thiosulfate enriched culture and non-enriched culture, respectively.

These equations and the *in situ* FDC values of bacterioplankton were used for estimating the *in situ* growth rates. With an average *in situ* FDC of 2.4%, the overall average *in situ* growth rate was calculated to be 0.32 hr^{-1} (generation time of 2.1 hrs), whereas the growth rate was 0.33 hr^{-1} (2.1 hrs) for the thiosulfate-enriched culture and 0.40 hr^{-1} (1.7 hrs) for the non-enriched culture. These estimations suggest that the thiosulfate-utilizers found near plune boundaries form a minority of the bacterioplankton community.

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北フィジー海盆の熱水プルーム内外における浮遊細菌の現存量と成長特性

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要旨：北フィジー海盆の熱水活動域における深層水から浮遊細菌を採集した。これらは熱水プルームの境界付近から採集され，浮遊細菌の現存量と成長速度をプルームの存在域と化学合成の観点から解析した。

細菌現存量はプルーム内ではプルーム外よりも高かった。しかし，細菌成長速度にはプルーム内外で明らかな差が見られなかった。また，チオ硫酸塩添加培地の使用によって算定される化学合成細菌の生息密度の低いことと，チオ硫酸塩の添加が現場浮遊細菌群集の成長速度に影響を及ぼさなかったことから，熱水プルームの境界付近に生息する浮遊細菌群集には，チオ硫酸塩利用の化学合成硫黄細菌の少ないことが明白となった。