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Occurrence and Distribution of Nitrogen-scavenging Bacteria in Marine Environment*

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The occurrence and distribution of nitrogen-scavenging bacteria in the water of coastal and oceanic regions of Japan were studied during the Seisui-Maru cruises from 1986 to 1987.

Nitrogen-scavenging bacteria in the water usually occurred at the level of 10－10^4 cfu/ ml. This value was almost comparable to that of aerobic heterotrophic bacteria.

It seems that nitrogen-scavenging bacteria play an important role in the efficient uptake of low levels of nitrogenous compounds in marine environment.

Key words : Nitrogen-scavenging bacteria, Marine bacteria, Kumano Nada, Nansei Shotō area.

The contents of nitrogenous compounds — organic form as well as inorganic form, are usually low in marine environment. Inorganic nitrogenous compounds as nutrient salts are sometimes one of the most important limiting factors for bioproduction in aquatic environment. The supply of inorganic nitrogenous compounds especially in surface waters is usually not enough for bacteria due to the competitive uptake of them with phytoplanktons. Molecular nitrogen, which is not taken up by phytoplanktons other than cyanobacteria, can be utilized as a nitrogen source by nitrogen-fixing bacteria. However, the process of nitrogen fixation requires a lot of energy. According to Bergey's Manual, *Azotobacter* and *Azomonas*, typical nitrogen-fixing bacteria in terrestrial environment, consume 1 g of carbohydrate for non-symbiotical fixation of at least 10 mg of atmospheric nitrogen. Therefore, nitrogen fixation is an enormous energy-consuming process from the viewpoint of biological energy economy. In general, the intensity of nitrogen fixation with a great energy demand depends

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primarily on the quantity of organic matter available (OLÁH *et al.*, 1983). The areas, where the organic matter necessary for nitrogen fixation abounds, are very few in marine environment : for example estuary, enclosed bay, sand beach, tideland, salt marsh and coastal eutrophied areas.

HILL *et al.* (1969) and JONES *et al.* (1980) reported that the microorganisms which can grow remarkably well on nitrogen-free media, have an ability to scavenge low levels of nitrogenous compounds from the atmosphere. JONES *et al.* (1980) also demonstrated that nitrogen-scavenging bacteria showed a high affinity for NH_4^+, compared with *Escherichia coli*. The nitrogen-scavenging bacteria in marine environment are very important specially in a nutrient poor environment (open sea) with low levels of available carbon (about 0.1 - 2.0 mg/l) and nitrogen (about 2 - 12 μg/l).

On the other hand, the concept of oligotrophic (oligocarbotrophic) bacteria was recently presented (KUZNETSOV *et al.*, 1979). The oligocarbotrophic bacteria were tentatively identified as those bacteria that can grow on media containing 1 - 15 mg organic-C/l and can be subcultured on such media (POINDEXTER, 1981). KADOTA and ISHIDA (1984) classified them into two groups : obligate oligotrophic bacteria and facultative oligotrophic ones. The facultative oligotrophic bacteria can grow on media containing 0.2 - 2 g of organic-C/l, while the obligate oligotrophic bacteria, on the other hand, can not (KADOTA and ISHIDA 1984). Therefore, some of nitrogen-scavenging bacteria may be considered as one of oligotrophic bacterial members. However, the nitrogen-scavenging bacteria don't always grow on organic-carbon poor media (about 1 - 15 mg C/l), although they show a high affinity for nitrogenous compounds in nitrogen poor media.

The present study was designated to obtain preliminary information concerning distribution of nitrogen-scavenging bacteria and their roles in energy economy from the viewpoint of nitrogen recovery into biological resource systems in marine environment.

Methods

Collection of water samples

Seawater samples were collected during the cruises of 86 - R - 9 and 87 - R - 3 by the Seisui-Maru of Mie University.

Figs. 1 and **2** show the location of sampling stations set up in coastal and pelagic regions of Japan.

Enumeration of bacterial number

Viable counts of aerobic heterotrophic bacteria and nitrogen-scavenging bacteria in the water were obtained by the smear plate technique. The medium (about 344 mg organic-C/l) for nitrogen-scavenging bacteria used in this study was composed of glucose, 0.20 g ; glycerin, 0.20 g ; mannitol, 0.20 g ; sodium acetate, 0.20 g ; sodium citrate, tribasic, 0.20 g ; yeast extract, 0.01 g ; NaCl, 30.0 g ; KCl, 0.7 g ; $MgCl_2 \cdot 6H_2O$, 10.8 g ; $MgSO_4 \cdot 7H_2O$, 5.4 g ; $CaCl_2 \cdot$

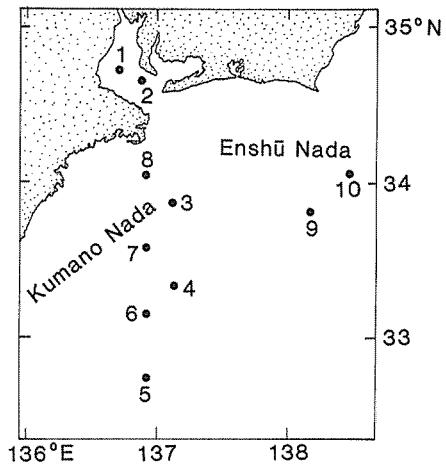

Fig. 1. Location of sampling stations set up in the Kumano Nada and Enshū Nada.

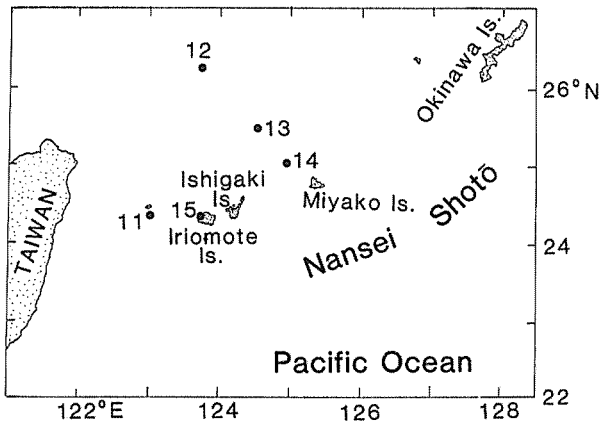

Fig. 2. Location of sampling stations set up in the Nansei Shotō area.

$2H_2O$, 1.0 g ; agar, 15.0 g and 1,000 ml distilled water with pH of 7.2 - 7.5. The medium for aerobic heterotrophic bacteria also consisted of polypepton, 5.0 g ; yeast extract, 5.0 g ; agar, 15.0 g and 1,000 ml seawater, pH 7.2 - 7.5. Cultivation was done at 25 ℃ for 1 - 2 weeks.

Results and Discussion

Water samples were collected from the Ise Bay and Kumano Nada areas during September 17 - 19, 1986. As shown in **Table 1**, nitrogen-scavenging bacteria in the surface water were abundant, i. e. 10^2 - 10^3 cfu/ ml. These values were comparable to those of aerobic heterotrophic bacteria. However, the occurrence of nitrogen-scavenging bacteria in the water column below 25 m were very few, ranging from 10 - 10^2 cfu/ ml.

As shown in **Table 2**, water samples were collected from the Nansei Shotō areas during May 12 - 14, 1987. The number of nitrogen-scavenging bacteria in the surface water of the Nansei Shotō areas ranged from 10^3 to 10^4 cfu/ ml. These values were sometimes more than those of aerobic heterotrophic bacteria.

Thus, nitrogen-scavenging bacteria are likely to distribute widespreadly in the water of marine environment.

HILL and POSTGATE (1969) described that putative nitrogen-fixing bacteria can not fix molecular nitrogen, but can scavenge low levels of nitrogenous compounds. KAWAI and SUGAHARA (1971a, 1971b, 1971c, 1971d) reported distribution of nitrogen-fixing bacteria which can grow on nitrogen-free media. Some of the nitrogen-fixing bacteria observed by KAWAI and SUGAHARA may include nitrogen-scavenging bacteria which cannot reduce acetylene to ethylene.

Table 1. Distribution of nitrogen-scavenging bacteria in the water of the Ise Bay, Kumano Nada and Enshū Nada.

Sampling station	Station depth (m)	Sampling date	Depth of water sample (m)	Water temp. (℃)	Aerobic heterotrophs (cfu/ml)	Nitrogen-scavenging bacteria (cfu/ml)
Ise Bay						
St. 1	29.7	Sep. 17, 1986	0	24.4	3.3×10^3	1.7×10^3
St. 2	26.7	Sep. 17, 1986	0	23.5	2.1×10^3	1.5×10^3
Kumano Nada and Enshū Nada						
St. 3	1,975	Sep. 17, 1986	0	23.1	2.4×10^3	1.5×10^3
St. 4	2,720	Sep. 18, 1986	0	27.2	1.8×10^3	2.4×10^3
St. 5	3,490	Sep. 18, 1986	0	27.8	2.0×10^3	2.6×10^3
			25			1.5×10
			50			2.5×10
			100			7.0×10
			200			1.1×10^2
St. 6	3,530	Sep. 18, 1986	0	28.1	2.1×10^3	4.3×10^3
			25			1.0×10
			50			1.0×10
			100			1.0×10
			200			1.0×10
St. 7	2,055	Sep. 18, 1986	0	23.5	2.2×10^3	2.5×10^3
			25			7.5×10
			50			3.0×10
			100			1.5×10
			200			1.0×10^2
			500			1.0×10
St. 8	813	Sep. 19, 1986	0	23.9	3.0×10^3	1.5×10^3
			25			4.5×10
			50			4.0×10
			100			3.5×10
			200			7.0×10
			500			4.0×10
St. 9	3,749	Sep. 19, 1986	0	24.0	2.2×10^3	1.9×10^3
St. 10	3,673	Sep. 19, 1986	0	22.9	2.2×10^3	5.9×10^2

Table 2. Distribution of nitrogen-scavenging bacteria in the water of the Nansei Shotō area.

Sampling station	Station depth (m)	Sampling date	Depth of water sample (m)	Water temp. (℃)	Aerobic heterotrophs (cfu/ml)	Nitrogen-scavenging bacteria (cfu/ml)
Nansei Shotō area						
St. 11	835	May 12, 1987	0	27.2	2.3×10^4	3.6×10^4
			25		4.0×10^2	2.2×10^2
			50			3.4×10^2
			100			6.9×10^2
			200			6.1×10^2
St. 12	145	May 13, 1987	0	26.8	1.2×10^4	2.5×10^4
St. 13	2,064	May 13, 1987	0	26.6	3.3×10^4	3.8×10^4
St. 14	1,790	May 14, 1987	0	25.5	1.1×10^4	8.1×10^3
			450			6.8×10^2
Kuira Estuary, Iriomote Island						
St. 15	2	May 12, 1987	0	25.3	1.7×10^3	3.0×10^3
			0	25.3	1.3×10^3	4.4×10^3
			0	25.3	4.7×10^3	3.6×10^3

Among nine DWW strains of WYNN-WILLIAMS isolated from Cardigan Bay of United Kingdom, 8 strains did not have the capacity to reduce acetylene to ethylene (JONES and RHODES-ROBERTS, 1980). According to JONES and RHODES-ROBERTS, non-nitrogen fixing strains were able to scavenge low levels of nitrogenous compounds mainly ammonia from the atmosphere. Eight strains of nitrogen-scavenging bacteria were all small gram-negative, motile rods with a respiratory oxidative metabolism and with orange-yellow pigments. One strain of them was *Pseudomonas* sp. Six strains were peritrichously flagellate. However, seven strains other than *Pseudomonas* sp. could not be further identified even to the genus level (JONES and RHODES-ROBERTS, 1980).

Little information on the generic composition of marine nitrogen-scavenging bacteria has been available to date. The authors collected more than 200 strains of nitrogen-scavenging bacteria from marine environment. Further studies on the taxonomical characteristics of marine nitrogen-scavenging isolates will be carried out.

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