水圏における窒素固定に関する微生物学的研究II.

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Microbiological Studies on Nitrogen Fixation in Aquatic Environments—II. On the Distribution of Nitrogen Fixing Bacteria in Sea Water Regions*

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By the use of the method reported in the previous paper, the distribution of nitrogen fixing bacteria was studied in some sea water regions. The standing crop of the bacteria in the water was 10^{-1}–10^{2} cells/mL in inland bays such as Maizuru Bay and Kumihama Bay in Kyoto Prefecture, while the number was quite small in offshore waters in Suruga Bay and Sagami Bay i.e. 0–800 cells/L.

In the water of East China Sea, the open sea, the standing crop of the nitrogen fixing bacteria was as small as 0–400 cells/L, which were detected mostly in the surface water above 100 m, and the bacterial number decreased rapidly with the depth below around 100 m.

Biological nitrogen fixation which transforms molecular nitrogen to ammonia or organic nitrogen, namely the process in which atmospheric nitrogen enters into biosphere, is one of the most important process in nitrogen cycle in aquatic environments.[1] The organisms capable of fixing molecular nitrogen are known to occur in various sea water regions[2–8], however, little information has been available about the quantitative distribution of nitrogen fixing bacteria.

In the present paper, an attempt was made to make clear the distribution of nitrogen fixing bacteria in various sea water regions.

Methods

Counting of bacterial number. The membrane filter counting method was used for the enumeration of nitrogen fixing bacteria and total heterotrophic bacteria in the sea water collected from East China Sea, Suruga Bay and Sagami Bay. The M.P.N. method[1] was used for counting of the bacteria in the sea water collected from Maizuru Bay and Kumihama Bay. The medium for the enumeration of nitrogen fixing bacteria was as follows: Sucrose 20.0 g, KH_2PO_4 0.2 g, $Na_2MoO_4 \cdot 2H_2O$ 0.01 g, $FeSO_4$ 0.001 g, $MnSO_4$ 0.001 g, Artificial sea water[9] 1,000 mL, pH 7.6–8.0. In the preparation of the medium,

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artificial sea water was used in order to keep the medium free from nitrogen compounds which may be derived from natural sea water. KH_2PO_4 was added into the medium aseptically after separately autoclaved. In the plate culture, purified agar (Difco) was added to the medium in the concentration of 15 g per 1 liter. The following medium, Medium PPES–II[10], was used for the enumeration of total heterotrophic bacteria in sea water samples using the membrane filter counting method: Polypeptone 2.0 g, Proteose-peptone No. 3 1.0 g, Bacto-soytone 1.0 g, Bacto-yeast extract 1.0 g, Ferric phosphate soluble 0.1 g, Agar 15.0 g, Marine mud extract 100 mL. Aged sea water 900 mL, pH 7.6–7.8. The following medium was also employed for counting the number of total heterotrophic bacteria by the M.P.N. method: Bacto-casitone 5.0 g, Beef extract 3.0 g, KH_2PO_4 0.1 g, KNO_3 0.5 g, Fe–EDTA 6.0 mg, Artificial sea water 1,000 mL, pH 7.6–8.0. KH_2PO_4 was added into the medium aseptically after separately autoclaved.

Measurements of chemical properties of water. pH value of the water was measured by the use of pH meter of Model HM–5A (Toa Electronics Ltd.). Dissolved oxygen in water samples was analyzed by using Winkler's method and C.O.D. value was estimated from the amount of $KMnO_4$ consumed under alkaline reaction. Ammonia was determined by the method of RICHARDS and KLETSCH[11], in which ammonia is oxidized to nitrite by sodium hypochlorite and the nitrite is determined colorimetrically. Nitrite was also determined colorimetrically by the use of Griess-Romjn reagent. Nitrate was determined by the method of STRICKLAND and PARSONS[12], in which nitrate in water is reduced to nitrite by percolating through Cd–Cu column and the nitrite formed is determined colorimetrically. Inorganic phosphate was determined by the method of MURPHY and RILEY[13].

Water regions investigated. The investigation was carried out in Maizuru Bay and Kumihama Bay as the representative of inland bays which are surrounded by the land. Suruga Bay and Sagami Bay were chosen as offshore regions and East China Sea as open sea.

Maizuru Bay is located in the north part of Kyoto Prefecture. It occupied the surface area of about 26 km^2 with the maximum depth of about 25 m. Two stations were set up in Maizuru Bay: Station D (about 15.0 m in the depth) is in the center of the bay, about 800 m off from the shore, and Station E (about 6.0 m in the depth) is near the shore.

Kumihama Bay is situated in the north part of Kyoto Prefecture and connected with Japan Sea through a shallow and narrow cannal with 300 m of the length, 30 m of the width and about 3 m of the depth. It has the surface area of about 7 km^2, the coastal length of 22 km and the maximum depth of 22 m. The bottom at the entrance with the depth of about 3 m is sandy in this bay. The depth of the north part in this bay is deeper than 10 m and the south part is shallow, at most 10 m in the depth. Most of the bottom muds are black mud with the smell of sulfide. Sampling station (Station α) was set up in the

center of the bay.

Suruga Bay and *Sagami Bay* lie at the center of the Main Island of Japan and face Pacific Ocean. Kuroshio current passes through in the offing of the bays. The depth in Suruga Bay is around 200–2,000 m and the bottom is mostly mudy. In Sagami Bay, the depth is also about 200–1,500 m, and the quality of the bottom is mostly mudy. Four stations were set up in Suruga Bay and Sagami Bay.

East China Sea is comparatively shallow area surrounded by Southwest Islands and China. It occupies the area of 1.249×10^6 km^2 with the volume of 2.35×10^5 km^3, and most of the area are covered with the continental shelf, the mean depth of 188 m. Stations 1, 2, 3 and 4 were set up in East China Sea, and Stations 5 and 6 in the area to the south from Ishigaki Island, and Stations 7, 9, 10 and 11 in the region to the east of Southwest Islands.

Collection of water samples. The sea water samples from East China Sea were collected on May 20–June 2, 1968 from Stations 1, 2, 3, 4, 5, 6, 7, 9, 10 and 11 on the cruise of KH 68–2 by R/V Hakuho-maru of the Ocean Research Insitute, the University of Tokyo. The samples from Suruga Bay and Sagami Bay were taken on October 10, 1968 from Station 1a, on October 11, 1968 from Station 2a, on October 13, 1968 from Station 3a and on October 14, 1968 from Station 4 on the cruise of KT 68–20 by R/V Tansei-maru of the same Institute. The samples from Kumihama Bay were collected on May 26, 1967 and August 22, 1967 at Station α by R/V Misaki-maru of Kyoto Prefectural Fisheries Station and the samples from Maizuru Bay were taken by the boat of Kyoto University on November 21, 1967 from Station D, on July 5, 1969 and July 18, 1969 from Station E.

The collection of water samples for chemical analysis was carried out with the Nansen's bottle or Kitahara's bottle, and the J–Z sampler[14] was used for collecting microbiological samples from the surface water in offshore and from various depths of water column in Kumihama Bay and Maizuru Bay. ORIT sampler[10] was used for collecting microbiological samples from various depths in offshore.

Results and Discussion

Maizuru Bay and Kumihama Bay. The quality of the water of Maizuru Bay was shown in Table 1 and that of Kumihama Bay in Table 2. In spring, there is no remarkable difference between the contents of inorganic nitrogenous compounds in the water of Maizuru Bay and those of Kumihama Bay. It was observed that the value of C.O.D. in the water of Kumihama Bay is rather high as compared with Maizuru Bay. The decline in oxygen tension in the water of Kumihama Bay results in anoxic or low-oxygen condition in the deep layer of the water during summer season.

The number of the bacteria in the water in these bays was shown in Table 3. The

results indicate that the number of total heterotrophic bacteria in Maizuru Bay is about 10^2–10^3 cells/mL and nitrogen fixing bacteria are always present although comparatively few, i.e. 10^{-1}–10^1 cells/mL in each depth of Maizuru Bay. The number of nitrogen fixing

Table 1. Quality of the water in Maizuru Bay.

Date	11/21, 1967			7/5, 1969	7/18, 1969
Station (Depth)	Station D (15 m)			Station E (6 m)	
Depth (m)	0	5	10	5	5
Water temp. (°C)	15.6	16.6	17.5	21.8	23.8
D.O. (O_2 mg/L)	6.69	7.09	6.73	——	——
pH	8.0	8.0	8.1	——	——
NH_4–N (μg atoms/L)	1.47	0.90	1.19	0.30	0.04
NO_2–N (μg atoms/L)	0	0.93	1.01	0.06	0.40
NO_3–N (μg atoms/L)	0.13	0	1.29	0	0.33
C.O.D. (O_2 mg/L)	0.64	0.64	0.32	6.89	1.48
PO_4–P (μg atoms/L)	4.35	3.48	3.48	3.16	4.74

Table 2. Quality of the water in Kumihama Bay.

Depth (m)	1	5	10	15	20
Water temp. (°C)	21.5	19.5	16.5	15.7	16.0
D. O. (O_2 mg/L)	7.18	8.66	2.78	2.10	2.00
pH	8.0	8.1	7.8	7.6	7.8
NH_4–N (μg atoms/L)	2.81	0.56	3.37	0.56	0.56
NO_2–N (μg atoms/L)	0.16	0.22	0.19	0.30	0.16
NO_3–N (μg atoms/L)	0.20	0.34	0.10	0	0.60
C.O.D. (O_2 mg/L)	4.12	4.76	2.78	1.40	4.04
PO_4–P (μg atoms/L)	0.7	0.6	1.8	2.1	3.2

Station α, 5/26, 1967

Table 3. Distribution of nitrogen fixing bacteria in the water of Maizuru Bay and Kumihama Bay.

Date	Bay	Station (Depth)	Depth of water (m)	Total heterotrophs (cells/mL)	Nitrogen fixing bacteria (cells/mL)
11/21, 1967	Maizuru Bay	Station D (15 m)	0	7.9×10^3	9.2
			5	2.2×10^3	2.1×10
			10	4.6×10^3	9.1
7/5, 1969		Station E (6 m)	5	——	1.7
7/18, 1969			5	1.3×10^2	0.4
5/26, 1967	Kumihama Bay	Station α (20 m)	1	3.3×10^4	1.3×10^2
			20	7.8×10^3	1.1×10^2
8/22, 1967			5	2.2×10^3	8.2×10
			15	3.3×10^4	1.8×10

bacteria came about 1/100 of total heterotrophic bacteria. In Kumihama Bay, total heterotrophic bacteria usually occurred at the level of 10^3–10^4 cells/mL at each depth. Nitrogen fixing bacteria in the water were abundant, i.e. 10^2 cells/mL both in the surface and in the bottom water in May, 1967 and the number decreased slightly to the extent of 10^1 cells/mL in August, 1967. It was observed that nitrogen fixing bacteria in the water of Kumihama Bay are usually abundant as compared with those of Maizuru Bay. The correlation between the number of nitrogen fixing bacteria and C.O.D. value was found in the water of such bays. The amount of organic matter in sea water may restricts the growth of nitrogen fixing bacteria.

Suruga Bay and Sagami Bay. The results in Table 4 showed that total heterotrophic bacteria are about 10^3–10^5 cells/L, but nitrogen fixing bacteria are very scanty, i.e. about 0–800 cells/L, as compared with water regions such as Maizuru Bay and Kumihama Bay.

Table 4. Distribution of nitrogen fixing bacteria in the water of Suruga Bay and Sagami Bay.

Station Date	Depth of Water (m)	Water temp. (°C)	Salinity (‰)	Total heterotrophs (cells/L)	Nitrogen fixing bacteria (cells/L)
Suruga Bay Station 1a 10/10, 1968	0	23.2	33.856	37,000	80
	40	18.67	34.530	14,000	60
	80	——	34.672	12,000	0
	125	——	34.544	19,000	0
	259	10.42	34.464	8,000	230
	681	4.45	34.375	———	10
Suruga Bay Station 2a 10/11, 1968	0	23.0	34.046	41,000	110
	46	21.33	34.300	30,000	20
	92	15.85	34.626	25,000	110
	180	12.56	34.518	20,000	30
	372	8.04	34.362	14,000	6
	981	3.29	34.522	8,000	20
	1,181	2.85	34.537	6,000	20
Sagami Bay Station 3a 10/13, 1968	0	21.5	33.971	550,000	160
	48	19.70	34.336	19,000	380
	99	15.13	34.589	24,000	680
	182	12.68	34.507	9,000	150
	354	8.86	34.346	10,000	120
	908	3.38	34.434	5,000	40
	1,115	2.99	34.478	9,000	50
Sagami Bay Station 4 10/14, 1968	0	21.6	33.788	43,000	270
	51	18.87	34.512	19,000	760
	101	14.78	34.643	20,000	40
	201	11.74	34.531	25,000	30
	404	7.67	34.337	11,000	6
	1,030	3.25	34.400	9,000	0

The number of nitrogen fixing bacteria was most abundant at 50–100 m layer, but extremely small in the water column at the depth of below 100 m. According to the data of the vertical distribution of salinity, water temperature and the bacteria, it seemed that there may be different kinds of water mass at the depth of 0–100 m and below 100 m. However, nitrogen fixing bacteria and total heterotrophic bacteria in each water mass were very poor in offshore regions as compared with those of inland bays. The vertical distribution of the bacteria seemed to have the tendency that the dense bacterial biomass is found usually only in the topmost productive zone at the depths from 0 to 100 m and the

Table 5–1. Distribution of nitrogen fixing bacteria in the water of East China Sea.

Depth of water (m)	Water temp. (°C)	Salinity (‰)	D. O. (mL/L)	Total hetero-trophs (cells/L)	Nitrogen fixing bacteria (cells/L)
Station 1: 31°48.0′N 125°01.2′E Depth 47 m, 5/20, 1968					
0	14.3	32.854	6.30	——	120
20	13.92	32.893	7.62	——	20
Station 2: 29°59.7′N 124°59.7′E Depth 58 m, 5/23, 1968					
0	16.46	34.256	5.62	——	20
19	15.45	34.256	5.97	——	0
47	14.02	34.423	5.67	——	210
Station 3: 28°00.3′N 125°00.5′E Depth 100 m, 5/24, 1968					
0	21.78	34.244	5.25	——	50
19	21.46	34.308	5.16	——	0
48	19.62	34.394	5.13	——	20
95	17.39	34.575	3.97	——	10
Station 4: 26°00.5′N 125°00.7′E Depth 1,700 m, 5/25, 1968					
0	26.17	34.625	4.67	——	150
21	26.10	34.629	4.74	——	6
51	25.77	34.656	4.71	——	70
95	24.27	34.780	4.81	——	0
169	21.09	34.802	4.34	——	40
Station 5: 23°59.2′N 124°58.8′E Depth 2,575 m, 5/26, 1968					
0	25.82	34.820	4.92	——	4
21	24.34	34.830	5.41	——	20
52	22.33	34.888	5.13	4,000	40
102	20.32	34.927	4.63	4,200	10
888	4.52	34.392	1.74	920	20

Table 5–2. Distribution of nitrogen fixing bacteria in the water of East China Sea.

Depth of water (m)	Water temp. (°C)	Salinity (‰)	D. O. (mL/L)	Total hetero-trophs (cells/L)	Nitrogen fixing bacteria (cells/L)
Station 6: 22°00.0′N Depth 5,960 m, 5/27, 1968 125°01.3′E					
15	26.93	34.714	4.72	——	70
70	23.48	34.866	4.90	——	160
122	22.06	34.915	4.76	——	20
1,015	4.06	34.489	2.08	——	8
2,041	2.00	34.645	2.83	——	2
4,078	1.60	34.692	3.35	——	10
Station 7: 21°59.8′N Depth 5,650 m, 5/29, 1968 132°01.0′E					
0	26.96	34.723	4.72	6,300	60
21	26.03	34.805	4.77	——	20
52	22.84	34.926	5.01	——	100
102	20.72	34.958	4.98	3,600	100
198	18.34	34.898	4.81	3,200	70
1,004	3.66	34.401	1.40	240	20
1,965	1.98	34.642	2.72	——	50
4,010	1.60	34.697	3.42	——	30
Station 9: 26°00.2′N Depth 3,050 m, 5/31, 1968 131°59.8′E					
0	23.35	34.709	4.94	——	100
17	23.00	34.705	4.94	4,200	4
42	20.85	34.915	5.26	1,300	40
83	19.13	34.925	4.79	2,900	4
161	17.87	34.889	4.88	5,500	0
1,066	3.44	34.451	1.66	360	30
2,159	1.90	34.653	2.83	——	30
Station 10: 28°00.2′N Depth 2,300 m, 6/1, 1968 132°00.0′E					
0	22.88	34.729	5.02	2,500	4
45	21.35	34.827	5.11	4,600	110
88	20.06	34.947	5.42	3,000	0
175	18.20	34.895	4.97	3,500	2
2,154	1.87	34.637	2.81	——	2
Station 11: 30°01.2′N Depth 3,700 m, 6/2, 1968 132°00.8′E					
0	24.32	34.594	4.93	——	380
22	24.29	34.613	4.87	——	8
56	22.95	34.629	4.74	——	220
112	21.31	34.840	4.36	——	60
168	19.18	34.862	4.43	——	40
224	17.88	34.836	4.51	——	30
439	11.59	34.442	3.68	——	20
1,860	2.08	34.611	2.57	——	8

number of the bacteria decreases rapidly with the depth below 100 m.

East China Sea. The results in Tables 5–1 and 5–2 showed that total heterotophic bacteria occur as many as about 0–10^3 cells/L and nitrogen fixing bacteria are very scanty in their number, at most 400 cells/L. The pattern of vertical distribution had the tendency that the dense bacterial biomass, i.e. 100 cells/L or sometimes more, occurs only in the topmost zone at the depths from 0 to 100 m and the number decreases rapidly with the depth to the extent of atmost several cells per 1 liter. From data of salinity, water temperature and distribution of bacteria, it was suggested that the water mass in the upper layer (0–100 m) is quite different from that below 100 m and nitrogen fixing bacteria are abundant in the former water (0–100 m) which is influenced by Kuroshio axis.

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References

1) A. KAWAI and I. SUGAHARA: This Bull., **37**, 740–746 (1971).
2) W. BENECKE and J. KEUTNER: *Ber. deut. bot. Gesell.*, **21**, 333–346 (1903).
3) W. BENECKE: *ibid.*, **25**, 1–7 (1907).
4) J. REINKE: *ibid.*, **21**, 371–380 (1903).
5) J. REINKE: *ibid.*, **21**, 481–483 (1903).
6) S. A. WAKSMAN, H. W. REUSZER, C. L. CAREY, M. HOTCHKISS, and C. E. RENN: *Biol. Bull.*, **64**, 183–205 (1933).
7) S. A. WAKSMAN, M. HOTCHKISS, and C. E. CAREY: *ibid.*, **65**, 137–167 (1933).
8) L. N. PSHENIN: in "Symposium on Marine Microbiology" (C. H. OPPENHEIMER ed.), 383–391, C. C. Thomas Publ., Springfield, Illinois (1963).
9) J. LYMAN and R. H. FLEMING: *J. Mar. Res.*, **3**, 134–146 (1940).
10) N. TAGA: *Bull. Misaki Mar. Biol. Inst., Kyoto Univ.*, **12**, 65–76 (1968).
11) F. A. RICHARDS and R. A. KLETSCH: *Bull. Fish. Res. Bd. Canada*, **125** (revised), 83–87 (1965).
12) J. D. H. STRICKLAND and T. R. PARSONS: *ibid.*, **125** (revised), 73–78 (1965).
13) J. MURPHY and J. P. RILEY: *Anal. Chem. Acta*, **27**, 31–36 (1962).
14) C. E. ZOBELL: *J. Mar. Res.*, **4**, 173–188 (1941).