Distribution of Cellulolytic Bacteria in the Sea of Hiuchi-Nada Area

Hideo MIYOSHI

Laboratory of Science of Aquatic Environment, Faculty of Agriculture

Abstract : Distribution of both anaerobic and aerobic cellulolytic bacteria was examined in the sea of Hiuchi-Nada area with special reference to possible organic nutrients and oxidation-reduction conditions. Both anaerobic and aerobic cellulolytic bacteria were scarce in seawater and relatively abundant in recent sediments. Thus the vertical distribution of cellulolytic bacteria is more likely to be influenced by the level of organic matter. Anaerobic cellulolytic bacteria were relatively predominant in the near-shore area where reducing conditions were prevalent, while aerobic cellulolytic bacteria were relatively predominant in the off-shore area where oxidizing conditions were prevalent. That is, the geographical distribution of anaerobic or aerobic cellulolytic bacteria seems to be affected by not only the level of organic matter but the intensity of redox potential. Both anaerobic and aerobic cellulolytic bacteria in the sea were more abundant in summer than in winter.

Introduction

In our country increasing "eutrophication" of coastal waters occurs, and contamination by the cellulosic matter which originates in plankton remains is becoming ubiquitous in coastal sediments. In such evironments aerobic decomposition of cellulose is rather limited owing to the concurrent development of reducing conditions, so the metabolic activity of marine anaerobic cellulolytic bacteria seems to be of ecological importance.

The data concerning the distribution of marine aerobic cellulolytic bacteria were given by Kadota[1], but little work on the distribution of marine anaerobic cellulolytic bacteria has been published.

The present study was undertaken to elucidate the distribution of anaerobic and aerobic cellulolytic bacteria in the sea with special reference to possible organic nutrients and oxidation-reduction conditions.

Materials and Methods

Sampling Water samples were collected with a Van Dorn sampler. Sediment samples were collected using a gravity corer equipped with a 4 × 20 cm diam. core tube. Aliquots of water and sediment samples were stored at 4°C, and subjected to bacteriological examinations within several hours after collection. Other aliquots of sediment samples were stored at −20°C just after collection, and subjected later to chemical examinations.

Enumeration of cellulolytic bacteria Viable cells of both anaerobic and aerobic cellulolytic bacteria were enumerated by the extinction dilution method ; hydrolysis of filter paper was adopted as the indicator of growth ; and most probable number (MPN) was obtained according to the system of three tubes for each dilution.

Cultivation of anaerobic cellulolytic bacteria by use of pre-reduced medium The pre-reduced medium contained; peptone (Difco), 0.5 g; NH_4Cl, 2 g; K_2HPO_4, 0.01 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; resazurin, 1 mg; cysteine $HCl \cdot H_2O$, 0.5 g; $Na_2S \cdot 9H_2O$, 0.5 g; Na_2CO_3, 4 g; and 1 liter of aged seawater. A strip of filter paper was added for each

cultivation vessel as carbon source. The techniques for preparing the medium and inoculating the sample under oxygen-free CO_2 were based on those described in the Anaerobe Laboratory Manual[2].

Cultivation of anaerobic cellulolytic bacteria by use of anaerobic jar The medium employed contained: peptone (Difco), 0.5 g; NH_4Cl, 2 g; K_2HPO_4, 0.01 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; resazurin, 1 mg; aged seawater, 1000 ml; (pH, 7.2). The medium was dispensed in 5 ml amounts in test tubes capped with cotton plugs, and enriched with a filter paper in each test tube. Just prior to inoculation filter-sterilized Na-ascorbate was added aseptically to give a final concentration of 0.5 g / liter.

Immediately after inoculation, two different incubation procedures were adopted to ensure the anaerobiosis of cultures. A series of inoculated tubes was placed in an anaerobic jar with a palladium-coated alumina pellet catalyst, and the jar was evacuated and refilled with oxygen-free CO_2 and H_2 in a ratio of 1 : 9. Another series of inoculated tubes was placed in an anaerobic jar with a chromium-sulfuric acid mixture, and the jar was evacuated and flushed with sterile H_2. As additional H_2 was generated in the jar, excess H_2 was released via a outlet tube dipping just beneath the surface of mercury.

Cultivation of aerobic cellulolytic bacteria A medium having the same composition as "Medium 7" proposed by Kadota[1] was employed.

Measurement of *Eh* values Immediately after sampling sediment, the platinum and reference electrode pair of a portable redox meter was inserted into the sample for 20 min., and redox potential (*Eh*) was measured. Therefore, *Eh* values obtained were probably higher than intrinsic values to some degree.

Chemical analyses of sediments Prior to chemical examinations of sediment, shell debris and very coarse sands larger than 1 mm in diameter were removed by hand from samples. Carbohydrates and total organic nitrogen were measured respectively in the same manners as described previously[3]. Total organic carbon was measured by procedures similar to those described by Menzel and Vaccaro[4] : the sample plus oxidizing agents were put into a glass ampule, organic carbon was oxdized to CO_2 by heating the sealed ampule to 175°C for 1 hr. Then evolved CO_2 was measured by a non-dispersive infrared gas analyzer. Cellulose was determined by the procedure described by Updegraff[5].

Results and Discussion

As a preliminary experiment, three different anaerobic culture methods were examined for their ability to detect anaerobic cellulolytic bacteria of marine origin. As shown in Table 1, the counts obtained by use of the pre-reduced media were consistently higher than those of the remaining two methods. Then, in both anaerobic jars some of the organisms seem to have lost their ability to proliferate before the redox potentials of the media were reduced low enough to initiate the growth of the organisms. But complete decolouration of resazurin in the media occurred within several hours after the jars were sealed. Therefore, most of marine anaerobic cellulolytic bacteria seem to be extremely oxygen-sensitive anaerobes-so-

Table 1. *Numbers of anaerobic cellulolytic bacteria detected by different enumeration methods*

St.	Core depth	Temp.	Anaerobic cellulolytic bacteria (MPN/g wet sediment)		
			Use of pre-reduced media	Use of cold catalyst jar	Use of chromium catalyst jar
A	0 – 1	16.7	480	46	18
	3 – 4	–	46	18	18
B	0 – 1	16.0	920	56	30
	3 – 4	–	186	30	30
C	0 – 1	16.5	520	86	86
	3 – 4	–	86	46	42
D	0 – 1	16.3	480	58	58
	3 – 4	–	186	22	18
E	0 – 1	16.0	480	86	86
	3 – 4	–	186	46	30

Note: Sediment samples were collected from the central area of Uranouchi Bay, Kochi Pref. (Apr. 19, 1974).

called fastidious anaerobes. Anyhow the use of pre-reduced medium gives the most satisfactory results for counting anaerobic cellulolytic bacteria of marine origin, and the conclusion is supported by the fact that there are many papers dealing with the superiority of a pre-reduced medium for cultivating fastidious anaerobes[2,6].

The abundance of cellulolytic bacteria was examined in relation to the character of their habitats in the Kawanoe and Saijo areas (Fig. 1). The Kawanoe area has been contaminated by effluents from pulp and paper manufacturing factories. On the other hand, the Saijo area has been contaminated by nutrient-rich pollutants from the factories of chemical companies, and both areas have been recognized as examples of "accelerated eutrophication" in marine environment.

As described in Table 2, cellulolytic bacteria were scarce in seawater : anaerobic cellu-

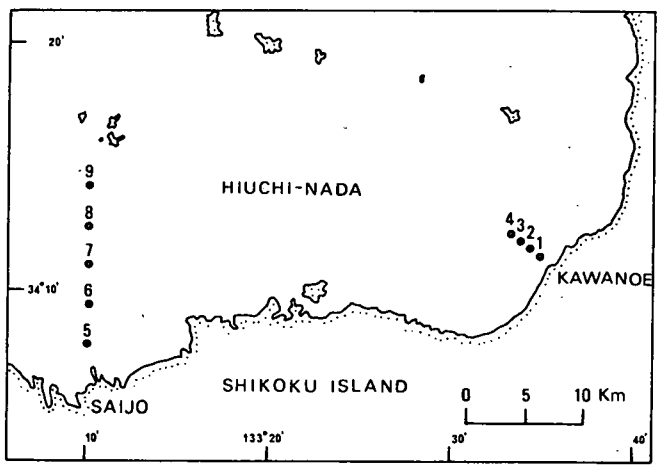

Fig. 1. Map of Hiuchi-Nada area, showing sampling stations.

Table 2. *Viable cells of cellulolytic bacteria in seawnter and some additional data on their environmental characteristics*

Date of sampling	St.	Depth of water (m)	Trans-parency (m)	Depth of sampling (m)	Temp. (°C)	Cellulolytic bacteria (MPN/ml) Anaerobic	Aerobic
19 Aug. '75	5	12	2.5	0	28.1	0.8>	2.3
				11	26.3	0.8	0.4>
	6	13	3.1	0	27.0	0.8>	2.3
				12	26.7	0.8>	0.4
	7	18	4.0	0	28.5	0.8>	0.4
				17	26.8	0.8>	0.4
	8	21	9.9	0	27.8	0.8>	0.4
				20	26.8	0.8>	0.4>
	9	33	6.9	0	27.4	0.8>	0.4
				32	26.8	0.8>	0.4>
7 Feb. '76	5	13	5.5	0	9.8	0.8>	0.4>
				12	9.8	0.8	0.4
	6	16	6.8	0	10.0	0.8>	0.4>
				15	10.1	0.8>	0.4>
	7	19	6.5	0	9.8	0.8>	0.4>
				18	9.9	0.8>	0.4>
	8	21	7.0	0	10.0	0.8>	0.4>
				20	10.0	0.8	0.4>
	9	33	6.5	0	9.2	0.8>	0.4>
				32	9.3	0.8>	0.4>

lolytic bacteria were occasionally found only in the bottom layers of water, and greater density of aerobic cellulolytic bacteria was found especially in the surface layers of water near shore in summer. The sparsity of cellulolytic bacteria in seawater seems to reflect the fact that the suspended matter which is available for cellulolytic bacteria can not remain so long in seawater.

As given in Table 3, the population range of cellulolytic bacteria within several centimeters of the surface of the sediment was 10^0-10^3 MPN/g wet sediment. Anaerobic cellulolytic bacteria in sediment were relatively dense near shore, and decreased with increasing distance from shore. The reverse was found for the geographical distribution of aerobic cellulolytic bacteria. Both anaerobic and aerobic cellulolytic bacteria in sediments were relatively dense within the uppermost few centimeters of sediments especially in summer.

The characteristics of their habitats are also given in Table 3. *Eh* values of sediments were relatively high at the surface of sediments especially in the off-shore area. Rough paralells were observed among the levels of total organic carbon and total organic nitrogen, and in most cases the levels of these contents were relatively large at the surface of sediment especially in near-shore sediments, and apparent seasonal variations were not observed. A definite pattern of spacial variation of cellulose in sediments was observed in Kawanoe area, but cellulose in sediment was almost evenly distributed in Saijo area so far as the present data were concerned. The spacial distribution of carbohydrates in sediments had rough paralells with total organic carbon or total organic nitrogen, but the concentration of carbohydrates in sediments was apparently low in winter.

Based on the above data, the contribution of environmental factors to the distribution of

Table 3. *Abundance of cellulolytic bacteria in sediments and some environmental characteristics of their habitats*

Date of sampling	Station	Depth of overlying water (m)	Core depth (cm)	Temp. (°C)	*Eh* (mV)	Cellulolytic bacteria (MPN/g wet mud)		Total organic carbon (mg C/g dry mud)	Carbohydrates (mg glucose/g dry mud)	Cellulose (mg glucose/g dry mud)	Total organic nitrogen (mg N/g dry mud)
						Anaerobic	Aerobic				
1 Mar. '75	1	14	0–0.5	11.4	+40	1900	93	16.6	7.6	2.05	1.46
			6–6.5	—	−60	480	20	7.8	1.6	0.38	0.78
	2	19	0–0.5	10.8	+120	920	43	11.6	2.5	0.41	1.20
			6–6.5	—	−50	86	11	9.2	1.5	0.23	1.03
	3	31	0–0.5	11.1	+260	920	120	11.1	2.5	0.40	1.25
			6–6.5	—	−50	110	4	9.6	1.3	0.15	1.15
	4	34	0–0.5	11.4	+260	480	2300	11.2	2.6	0.26	1.33
			6–6.5	—	−90	86	23	10.1	2.5	0.22	1.22
19 Aug. '75	5	12	0–0.5	26.3	−40	920	93	11.1	3.0	0.20	1.32
			6–6.5	—	−60	480	11	9.2	2.0	0.19	1.13
	6	13	0–0.5	25.9	+90	180	150	9.8	2.5	0.18	1.16
			6–6.5	—	−90	46	4	8.2	1.9	0.19	1.01
	7	18	0–0.5	26.1	+280	190	460	8.0	2.3	0.20	0.96
			6–6.5	—	+210	86	43	6.9	1.9	0.19	0.86
	8	21	0–0.5	26.1	+320	190	460	7.7	2.0	0.22	0.88
			6–6.5	—	+220	46	15	7.9	1.8	0.20	0.92
	9	33	0–0.5	26.2	+320	190	460	7.2	1.8	0.21	0.84
			6–6.5	—	+260	86	43	6.2	1.6	0.18	0.72
7 Feb. '76	5	13	0–0.5	9.9	—	300	43	11.4	2.2	0.20	1.32
			6–6.5	—	—	32	4	9.5	1.6	0.19	1.10
	6	16	0–0.5	10.2	—	150	93	9.6	1.8	0.19	1.14
			6–6.5	—	—	9	15	8.7	1.4	0.19	1.00
	7	19	0–0.5	10.2	—	86	460	8.7	1.7	0.20	1.02
			6–6.5	—	—	9	4	7.4	1.2	0.18	0.86
	8	21	0–0.5	9.5	—	86	930	8.4	1.6	0.19	1.00
			6–6.5	—	—	18	3	7.3	1.2	0.18	0.86
	9	33	0–0.5	8.9	—	86	240	7.6	1.4	0.20	0.95
			6–6.5	—	—	7	7	6.4	1.1	0.17	0.76

cellulolytic bacteria in the sea can be summarized as follows : the vertical distribution of both anaerobic and aerobic cellulolytic bacteria seems to be affected by the level of organic matter, and geographical distribution of anaerobic or aerobic cellulolytic bacteria seems to be influenced by oxidation-reduction conditions in addition to the levels of organic matter ; anaerobic cellulolytic bacteria were predominant in near-shore sediment where reducing conditions were prevalent, on the contrary, aerobic cellulolytic bacteria were predominant in off-shore sediments where oxidizing conditions were prevalent.

In spite of increasing phytoplankton production, significant increase of cellulose content in recent sediments was not found off Saijo. The phenonenon is satisfactorily explained by the fact that a relatively large number of cellulolytic bacteria were distributed both in the water and the sediment of the area, and plankton remains resulted in the increase of organic matter in sediment without heavy contamination by cellulose. On the contrary, marked increase of cellulose content in recent sediment was encountered off Kawanoe. The interpretation is that a continuous discharge of cellulose to the area occurred throughout the seasons. Furthermore, the amount of cellulose in the effluents seems to be beyond a level capable of decomposition by the indigenous bacterial populations, and in the area, sediment is apt to be contaminated heavily by this cellulose.

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