浦の内湾における好気性従属栄養細菌と生物遺骸の分解

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Aerobic Heterotrophic Bacteria and the Decomposition of Dead Organisms in Uranouchi Bay

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To elucidate the involvement of bacteria in the decomposition of dead organisms in Uranouchi Bay, suspended matter, newly-formed deposits, and bottom mud samples were collected from the bay 4 times in 1977. Chemical and bacteriological examinations of these samples were made. The content of organic components decreased in the following order: suspended matter, newly-formed deposits, bottom muds. The levels of organic components in these samples were high in warm water months. Aerobic heterotrophic bacteria decreased in the order: newly-formed deposits, suspended matter, bottom muds.

In the marine environments, decomposition of dead organisms begins mostly in surface water, and further decomposition proceeds during the sinking process and after precipitation to the bottom floor. Free-living bacteria in the marine environment do not seem to be responsible directly for the decomposition because of the rapid dilution of their exoenzymes into surrounding water. Therefore, the following 3 groups of bacteria seem to be most important for the decomposition: (a) bacteria which are embedded in suspended matter; (b) those which are colonized on sinking particles; (c) those which inhabit surface bottom muds. To study this, the abundance and character of aerobic heterotrophic bacteria corresponding roughly to the above-described 3 groups were examined in Uranouchi Bay. At the same time, organic analyses of their habitats were carried out. The data obtained provide some information concerning the bacterial contribution to the decomposition of particulate organic matter in the marine environment.

Materials and Methods

Sampling Station

The station which was sampled throughout the present study is in the central part of Uranouchi Bay, as shown in Fig. 1. The sampling station was selected in the sea area having little influence of effluents and discharges from surrounding land or contamination from aquaculture farms. The bay is characterized, as a whole, by shallow turbid nutrient-rich waters. Results of oceanographic surveys over whole area of the bay have been previously reported.[1)]

Collection of Suspended Matter

Seawater was collected using a plastic type B sampler (Rigosha & Co. Ltd., Tokyo). An aliquot portion (2 or 3 *l*) of the seawater was filtered through a platinum-coated metallic mesh filter (Toyo Roshi Kaisha, Ltd., Osaka) having an advertised pore size of 10 μm. The particulate matter retained on the filter was employed for bacteriological examinations. Almost complete resuspension of particulate matter from the filter could be achieved by vigorous mixing and brushing. Great care was taken to ensure that no microbial contaminations occurred throughout the sampling and subsequent treatments. For chemical examinations, the metallic filter was replaced by a Reeve Angel 984H glass fiber filter.

Collection of Newly-formed Deposits

A 2-liter plastic bottle having an opening of 4.5 cm^2 was allowed to collect sedimenting matter at about 1 m above the bottom for 2 days. Solid matter which settled in the bottle was harvested aseptically by centrifugation at 4000 rpm for 5 min, and the precipitate was considered as newly-formed deposit.

Collection of Bottom Muds

Bottom sediment was taken using a gravity

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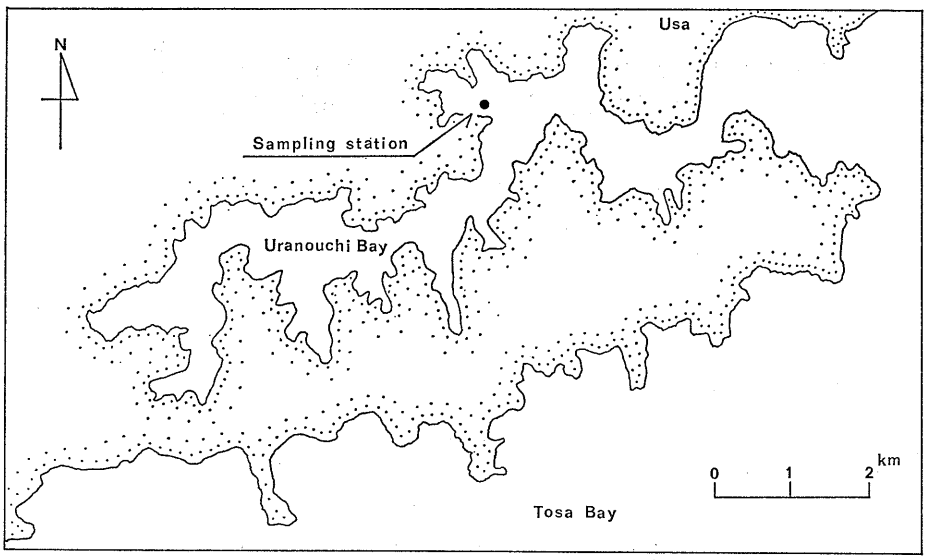

Fig. 1. Location of sampling station in Uranouchi Bay, Kochi Prefecture.

corer equipped with a 4×40 cm plastic core tube, and only the uppermost portion of core (0–5 mm) was used as a bottom mud sample. The mud obtained at this station largely consisted of silt.

Chemical Analyses

Organic carbon was measured by the procedures described by MENZEL and VACCARO.[2] Organic nitrogen was determined by the micro-KJELDAHL method with ninhydrin finish.[3] Proteins were determined by the ninhydrin method after being hydrolized with 6 N HCl at 100°C for 24 h in a sealed tube.[3] Carbohydrates were determined by the anthrone method.[3] Lipids were measured by a modified procedure of MUKERJEE.[3]

Bacteriological Examinations

In order to disperse bacteria from solid substrates into saline water, vigorous mixing for 2 min by a vortex mixer was employed. The counting of aerobic heterotrophic bacteria was carried out by the spread plate method of BUCK and CLEVERDON,[4] but the plating medium was replaced by a modified ZOBELL's 2216 E agar medium (final pH 7.5), consisting of Bacto-peptone, 5 g; Bacto-yeast extract, 1.0 g; K_2HPO_4, 0.01 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; Bacto-agar, 12 g; and aged seawater, 1 *l*. Bacteria were isolated randomly from high dilution plates. After purity was checked both microscopically and culturally, the isolates were subjected to the following examinations using a modified ZOBELL's 2216 E broth consisting of the above-described components minus agar as a basal medium. All cultures were incubated at 25°C. Gelatin hydrolysis was tested in medium containing 20% gelatin. After 2-weeks' incubation, the cultures were refrigerated at 5°C. Failures to solidify indicated hydrolysis. Casein hydrolysis was examined on plates containing 1% casein and 1.2% agar. After 4- to 7-days' cultivation, the plates were examined. Zones of clearing around colonies provided evidence for hydrolysis. Acid production from glucose was tested using broth containing 0.001% bromothymol blue and 0.5% glucose. After 3-days' incubation, acid production was detected by the colour change of bromothymol blue. In this study the tubes which showed a slight colour change (yellowish green) were also classified as positive tubes. Chitin digestion was determined according to the procedure described by OKUTANI.[5] Starch hydrolysis was examined on plates containing 0.5% soluble starch and 1.2% agar. After 4-days' incubation, hydrolysis was detected by flooding conventional LUGOL's solution onto the plates. The hydrolysis of tributylin was tested on plates containing 1.2% agar and 0.5% tributylin. After 4-days' incubation, hydrolysis was detected by the zones of clearing around colonies.

Results and Discussion

Suspended matter, newly-formed deposits, and bottom mud samples from Uranouchi Bay were examined chemically and bacteriologically in February, May, August, and November, 1977.

The contents of organic carbon, organic nitrogen, and major organic nutrients, such as carbohydrates, proteins, and lipids, in these 3 samples are shown in Tables 1, 2, and 3. Among the 3 kinds of samples, suspended matter had the highest content of each organic component consistently, and the bottom muds the lowest. Except for a few extreme cases, the levels of organic components were relatively low in February. The C: N ratio was decreased roughly in the order: suspended matter, newly-formed deposits, bottom muds. The ratios of carbohydrates to organic carbon or nitrogen, proteins to organic carbon or nitrogen, lipids to organic carbon or nitrogen were decreased roughly in the order: suspended matter, newly-formed deposits, bottom muds. But the ratios of carbohydrates to proteins, lipids to proteins, carbohydrates to lipids, and proteins to lipids showed no regular tendency among these 3 kinds of samples. Namely, the proportion of refractory material(s) which seemed to be other than carbohydrate, protein, or lipid, tended to increase in the marine environment, as the decomposition of dead organisms proceeded.

Table 1. Chemical analyses of suspended matter samples

Date (1977)	Depth of sampling (m)	Temp. (°C)	Conc. of suspended matter (mg dry matter/ *l*)	Water content (%)	Organic carbon (mg/g dry matter)	Organic nitrogen (mg/g dry matter)	Carbohydrates (mg glucose/g dry matter)	Proteins (mg glutamic acid/g dry matter)	Lipids (mg stearic acid/g dry matter)	C: N ratio
Feb. 2	0.5	11.2	2.3	67.6	110	18	65	120	85	6.1
	11.0	10.2	3.8	59.3	79	11	45	100	37	7.2
May 14	0.5	20.6	2.0	86.7	240	36	90	360	105	6.7
	12.5	18.4	4.9	86.5	90	15	29	82	24	6.0
Aug. 8	0.5	29.4	1.8	83.0	330	37	140	470	120	8.9
	11.0	28.8	1.1	90.1	340	47	130	650	205	7.2
Nov. 12	0.5	21.6	1.2	87.4	330	42	83	400	125	7.8
	12.5	22.0	1.4	80.6	240	36	79	360	115	6.7

Table 2. Chemical analyses of newly-formed deposit samples

Date (1977)	Depth of sampling (m)	Water content (%)	Deposition rate (g dry matter/ m^2/day)	Organic carbon (mg/g dry matter)	Organic nitrogen (mg/g dry matter)	Carbohydrates (mg glucose/g dry matter)	Proteins (mg glutamic acid/g dry matter)	Lipids (mg stearic acid/g dry matter)	C: N ratio
Feb. 4	11.0	63.0	63	20.0	2.6	8.2	24.7	6.4	7.7
May 16	12.5	70.0	108	19.6	2.8	6.3	19.9	6.8	7.0
Aug. 10	11.0	72.8	81	32.1	4.1	11.3	34.2	12.6	7.8
Nov. 14	12.5	51.0	427	19.2	2.2	4.5	16.6	2.8	8.7

Table 3. Chemical analyses of bottom mud samples

Date (1977)	Depth of overlying water (m)	Temp. (°C)	Water content (%)	Organic carbon (mg/g dry matter)	Organic nitrogen (mg/g dry matter)	Carbohydrates (mg glucose/g dry matter)	Proteins (mg glutamic acid/g dry matter)	Lipids (mg stearic acid/g dry matter)	C: N ratio
Feb. 2	12.0	10.7	52.5	13.2	1.3	3.1	8.6	3.9	10.1
May 14	13.5	17.5	53.8	13.8	1.4	2.3	7.8	3.1	9.8
Aug. 8	12.0	27.3	54.1	15.2	1.8	6.3	9.8	4.0	8.4
Nov. 12	13.6	22.3	45.8	10.8	1.2	1.8	7.3	3.2	9.0

The viable counts of aerobic heterotrophic bacteria for the 3 kinds of samples are given in Table 4. The counts for the suspended matter were on average one log lower than for the newly-formed deposits. The counts showed no consistent trend between sampling depths. Low counts for the suspended matter are not interpreted as diminishing the importance of detrital particles as bacterial habitats, because the suspended particles contain mostly actively growing plankton which seem to be free from bacteria.[6] The counts for the newly-formed deposits were high on the average, and the counts for the mud samples were consistently lower than those for the suspended matter or newly-formed deposits. As might be expected, lower counts for the suspended matter and newly-formed deposits were encountered in February, but the count for the mud sample had little seasonal variation. As a trial to evaluate organic nutrient condition, the indices of "number of bacteria loaded per unit weight of organic carbon" were calculated for the newly-formed deposits and bottom muds. The indices were converged to 10^7–10^8 c.f.u./mg C for the newly-formed deposits and 10^5–10^6 c.f.u./

Table 4. Viable counts of aerobic heterotrophic bacteria in the suspended matter, newly-formed deposits, and bottom mud samples

Date (1977)	Depth of sampling (m)	Bacterial count (c.f.u./g wet matter)		
		Suspended matter	Newly-formed deposits	Bottom muds
Feb. 2 / 4	0.5	8.3×10^6		
	11.0	5.6×10^6	8.7×10^7	
	12.0			1.4×10^6
May 14 / 16	0.5	2.0×10^7		
	12.5	1.8×10^6	1.1×10^5	
	13.5			5.1×10^6
Aug. 8 / 10	0.5	3.7×10^7		
	11.0	5.1×10^7	8.4×10^5	
	12.0			1.5×10^6
Nov. 12 / 14	0.5	3.7×10^7		
	12.5	6.7×10^7	1.2×10^5	
	13.6			8.9×10^5

Table 5. Relative abundance of aerobic heterotrophic bacteria having various biochemical abilities

Sample	Depth of sampling (m)	Sampling date (1977)	Hydrolytic activity of:					
			Gelatin (%)	Casein (%)	Tributyrin (%)	Glucose (%)	Starch (%)	Chitin (%)
Suspended matter	0.5	Feb. 2	62	62	52	96	60	30
	0.5	May 14	86	86	84	90	72	20
	0.5	Aug. 8	68	44	98	88	14	14
	0.5	Nov. 12	75	60	94	58	56	28
	11.0	Feb. 2	66	68	78	87	52	14
	12.5	May 14	84	76	80	82	46	8
	11.0	Aug. 8	82	72	98	94	28	56
	12.5	Nov. 12	74	70	94	78	55	45
Newly-formed deposits	11.0	Feb. 4	88	84	60	88	62	50
	12.5	May 16	92	84	96	98	74	54
	11.0	Aug. 10	58	48	98	86	28	4
	12.5	Nov. 14	74	66	86	84	58	44
Bottom muds	12.0	Feb. 2	74	76	22	76	64	62
	13.5	May 14	76	64	94	88	44	6
	12.0	Aug. 8	86	88	96	90	38	8
	13.6	Nov. 12	76	68	94	78	54	28

mg C for the bottom muds. This suggested that organic matter in the bottom muds was less favourable as a source of bacterial nutrients.

The biochemical ability to hydrolyze a variety of organic substances such as gelatin, casein, tributylin, glucose, starch, and chitin was examined for all the isolates. The results summarized as the relative abundance of bacteria having various biochemical ability in Table 5. There were a few extremely high or low values in these data, and, with a few exceptions, half or more of the bacteria from each sample were able to hydrolyze gelatin, tributylin, casein, and glucose; about half of the isolates could hydrolyze starch; less than half of them could attack chitin. In August, the proportion of bacteria which could hydrolyze tributylin was generally high, and that of bacteria which could hydrolyze starch was generally low, but there were no significant seasonal variations in the relative abundance of bacteria which could hydrolyze chitin, gelatin, casein, or glucose.

As a whole, the proportions of bacteria which were able to attack various organic substances were somewhat higher than previously reported.[7,8] This may reflect the fact that Uranouchi Bay is shallow and rich in organic nutrients.

The data on biochemical ability showed no clear trend sufficient to make a generalization on the relationship between the kind of sample and the biochemical character of attendant bacteria.

The method for collecting various stages of decaying organisms from marine environment had not been well established, and the selection of mesh size of filter used for collecting suspended matter needs further study.

In addition, the problems concerning the resuspension and advection of sedimented matter in the bay also need further study.

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