Distribution and Generic Composition of Lytic Enzyme-Producing Bacteria in Coastal and Oceanic Bottom Sediments^{*1, 2}

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Distribution and generic composition of lytic enzyme-producing bacteria in coastal and pelagic bottom sediments of Japan were studied.

Bacteria capable of producing lytic enzymes were in the range of 10^1 – 10^4 cfu per 1 g of wet bottom muds. The average percentage of the number of lytic enzyme-producing bacteria to that of heterotrophic ones was 12.1%.

Out of 482 strains of lytic enzyme-producing bacteria isolated from bottom sediments of Ise Bay, Matoya Bay, Owase Bay, Kumano Nada, Nansei Shotō and the surrounding area, *Bacillus* (87.3%) was the most dominant bacterial genus.

Microorganisms capable of producing bacteriolytic enzymes seem to be distributed widely in marine environment.

Nair *et al.*¹⁾ reported that the percent of bacteriolytic strains in the seawater isolates was 24–52% at Tokyo, Sagami and Suruga Bays of Japan. The percentage of bacteriolytic strains was higher in the bacteria isolated from zooplankton than those from seawater.¹⁾ A lytic bacterial population forms colonies on fresh organic particles more than detrital matter that have been subjected to initial decomposition process.¹⁾ They suggested that bacteriolytic enzymes may contribute to the initial decomposition of organic matter.¹⁾

Little information on the ecology of lytic enzyme-producing bacteria in marine bottom muds has been available to date.

The present paper deals with the distribution and generic composition of lytic enzyme-producing bacteria in neritic and pelagic bottom sediments.

Methods

Collection of Bottom Sediment Samples

The bottom sediment samples from Ise Bay, Kumano Nada, Nansei Shotō and the surrounding area were collected during the cruises of 83-R-2, 86-R-3 and 86-R-9 by the Seisui-Marui of Mie University.

Fig. 1 shows the location of sampling stations.

Enumeration of Bacterial Number

The opaque agar plate medium which contains *Micrococcus luteus* cells was used for counting the marine bacteria capable of lysing the bacterial cells. The medium used in this study was composed of polypepton (Daigo Eiyō Kagaku), 5.0 g; yeast extract (Nakarai Chemicals), 5.0 g; *Micrococcus luteus* cells (Seikagaku Kōgyō), 1.0 g (dry weight); agar, 15.0 g; 100 ml tap water and 900 ml seawater, pH 7.0. Bottom sediment samples were diluted to 10 – 10^4 times with sterilized aged seawater, and aliquots of 0.1 ml from each dilution was spread on the surfaces of opaque agar plates. All plates inoculated were incubated at 25°C for 2–7 days. The bacteria capable of lysing bacterial cells formed the transparent zone around the colonies on the opaque agar medium containing heat-killed cells of *Micrococcus luteus*.

Identification of Bacterial Strains

Four hundred eighty-two colonies were isolated randomly from opaque agar plates for counting bacteriolytic bacteria. The bacteriolytic bacterial isolates were purified by streaking on opaque agar plates containing *M. luteus* heat-killed cells. The generic composition of the isolates was determined according to the scheme of Simidu²⁾ and to Bergey's Manuals.^{3–5)}

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Fig. 1. Location of sampling stations set up in coastal and oceanic regions of Japan.

Table 1. Distribution of lytic enzyme-producing bacteria in the bottom sediments of Kumano Nada and its surrounding area

Sampling station	Station depth (m)	Sampling date	Sediment depth (cm)	Heterotrophs (cfu/g)	Bacteriolytic bacteria (cfu/g)
Ise Bay St. 1	29.7	Sep. 17, 1986	0-3	4.0×10^4	2.8×10^3 (7.0%)
			3-6	5.7×10^4	3.3×10^3 (5.8%)
			6-9	5.4×10^4	2.2×10^3 (4.1%)
			9-12	1.5×10^4	7.0×10^2 (4.7%)
			12-15	8.0×10^3	1.0×10^2 (1.3%)
			15-18	9.0×10^3	1.0×10^2 (1.1%)
			18-21	3.8×10^3	5.0×10 (1.3%)
			21-23	3.3×10^3	5.0×10 (1.5%)
St. 2	32.3	Sep. 17, 1986	0-2	3.4×10^4	2.7×10^3 (7.9%)
			2-4	3.1×10^4	1.9×10^3 (6.1%)
			4-6	2.6×10^4	1.2×10^3 (4.6%)
			6-8	1.7×10^4	7.0×10^2 (4.1%)
			8-11	3.1×10^4	1.5×10^3 (4.8%)
			11-14	2.0×10^4	8.5×10^2 (4.3%)
Matoya Bay St. 3	9.0	Sep. 13, 1973	0-3	2.2×10^5	2.3×10^4 (10.5%)
		Nov. 22, 1973	0-3	2.1×10^5	2.4×10^4 (11.4%)
Owase Bay St. 4	11.0	Aug. 23, 1973	0-3	2.5×10^5	1.2×10^4 (4.8%)
Kumano Nada and its surrounding area St. 5	1,975	Sep. 17, 1986	0-2	8.9×10^3	1.3×10^3 (14.6%)
			2-4	1.2×10^4	1.5×10^2 (1.3%)
			4-7	4.0×10^4	1.1×10^3 (2.8%)
			7-10	5.1×10^3	5.5×10^2 (10.8%)
			10-13	5.0×10^3	5.0×10^2 (10.0%)
			13-16	3.9×10^3	1.0×10^2 (2.6%)
			16-19	4.5×10^3	5.0×10 (1.1%)
			19-22	1.7×10^3	2.5×10^2 (14.7%)
St. 6	3,270	Apr. 24, 1983	0-3	2.1×10^4	2.5×10^3 (11.9%)
St. 7	3,490	Sep. 18, 1986	0-3	1.3×10^4	5.0×10 (0.4%)
St. 8	3,781	Sep. 19, 1986	0-2	2.3×10^4	1.5×10^3 (6.5%)
			2-5	1.6×10^4	2.0×10^3 (12.5%)
			5-8	1.9×10^4	1.5×10^3 (7.9%)
			8-11	1.8×10^4	1.6×10^3 (8.9%)

Values in parentheses show the percentage of the number of lytic enzyme-producing bacteria to that of heterotrophic bacteria.

Results

Distribution of Lytic Enzyme-Producing Bacteria

As shown in Table 1, heterotrophic bacteria usually occurred at the level of 10^3 – 10^5 cfu per

1 g of wet bottom sediment, but they were more abundant in enclosed bays, such as Ise, Matoya and Owase Bays. Bacteria capable of producing lytic enzymes were in the range of 10^1 – 10^4 cfu/g in Kumano Nada and the surrounding area.

Table 2. Distribution of lytic enzyme-producing bacteria in the bottom sediments of the Nansei Shotō area

Sampling station	Station depth (m)	Temp. of bottom water (°C)	Sampling date	Sediment depth (cm)	Heterotrophs (cfu/g)	Bacteriolytic bacteria (cfu/g)
St. 9	533	8.0	May 10, 1986	0–3	1.1×10^3	4.0×10^2 (36.4%)
St. 10	679	6.5	May 10, 1986	0–3	3.8×10^3	6.0×10^2 (15.8%)
St. 11	2,263	1.8	May 10, 1986	0–2	5.4×10^3	8.0×10^2 (14.8%)
				2–5	3.1×10^3	1.0×10^3 (32.3%)
				5–8	1.7×10^3	7.5×10^2 (44.1%)
				8–11	9.5×10^2	2.5×10^2 (26.3%)
				11–14	9.5×10^2	1.0×10^2 (10.5%)
				14–17	4.5×10^3	ND*
				17–20	5.0×10^3	ND*
				23–27	1.5×10^2	ND*
				27–33	5.0×10^3	ND*
St. 12	2,875	1.5	May 9, 1986	0–2	4.2×10^3	1.1×10^3 (26.2%)
				2–5	2.6×10^3	7.0×10^2 (26.9%)
				5–8	3.8×10^3	1.3×10^3 (34.2%)
				8–14	7.0×10^2	3.5×10^2 (50.0%)

Values in parentheses show the percentage of the number of lytic enzyme-producing bacteria to that of heterotrophic bacteria.

ND*: Not detected.

Table 3. Morphological and physiological characteristics of the lytic enzyme-producing bacteria isolated from bottom sediments of coastal and pelagic regions of Japan

	Ise, Matoya and Owase Bays	Kumano Nada area	Nansei Shotō area	Total
Number of strains tested	328	80	74	482 (100.0%)
Form rods	320	80	74	474 (98.3%)
cocci	8	0	0	8 (1.7%)
Gram positive	321	77	65	463 (96.1%)
negative	7	3	9	19 (3.9%)
Flagella polar	1	0	0	1 (0.2%)
lateral	10	1	1	12 (2.5%)
peritrichous	288	78	62	428 (88.8%)
Spore formation	304	76	41	421 (87.3%)
Catalase positive	328	80	74	482 (100.0%)
Oxidase positive	297	80	72	449 (93.2%)
Pigmentation	30	12	6	48 (10.0%)
Indole formation	0	0	0	0 (0%)
M. R. positive	114	22	39	175 (36.3%)
V. P. positive	232	66	17	315 (65.4%)
Nitrate reduction	147	51	45	243 (50.4%)
Denitrification	2	0	0	2 (0.4%)
Growth in 0% NaCl	318	75	47	440 (91.3%)
Heat resistance (100°C, 30 min)	304	76	41	421 (87.3%)
Starch hydrolysis	124	34	12	170 (35.3%)
Gelatin liquefaction	317	80	35	432 (89.6%)
Growth at 5°C	60	27	5	92 (19.1%)

Table 2 shows the distribution of lytic enzyme-producing bacteria in the bottom sediments of the Nansei Shotō area. The number of bacteria capable of lysing bacterial cells was 10^2 – 10^3 cfu per 1 g of wet bottom sediment. There was no distinct tendency of the vertical distribution of heterotrophic bacteria and lytic enzyme-producing ones in the sediment column.

The percentage of the number of lytic enzyme-producing bacteria to that of heterotrophic bacteria ranged from 0.4 to 14.7% in the Kumano Nada and its surrounding area, and from 10 to 50% in the Nansei Shotō area. The average percentage of bacteriolytic strains was 12.1%.

Generic Composition of Lytic Enzyme-Producing Bacteria in Bottom Sediments

Out of 482 strains of lytic enzyme-producing bacteria, 328 strains were isolated from enclosed bays (Ise, Matoya and Owase Bays), 80 strains from Kumano Nada and its surrounding area, and 74 strains from Nansei Shotō area.

As shown in Table 3, the majority of the isolates were spore-forming, gram positive rods with peritrichous flagella. About 91.3% of the isolates did not require NaCl for growth. About 89.6% of the bacterial strains are capable of liquefying gelatin and 35.3% can hydrolyse starch.

Table 4 lists the generic composition of lytic enzyme-producing bacteria isolated from coastal and oceanic bottom sediments. *Bacillus* was the most dominant bacterial genus in the bottom sediments. An unknown bacterial group which is gram positive, asporogenous motile rod with mainly peritrichous flagella comprised 7.1% of the isolated lytic enzyme-producing bacteria. Most of them have been considered as members of *Brevibacterium*. However, according to Bergey's Manual,⁵⁾ *Brevibacterium* is a gram positive, asporogenous non-motile aerobic bacterium with

a marked rod-coccus cycle. Further study is necessary in order to demonstrate taxonomical characteristics of an unknown bacterial group isolated during this study.

Discussion

Johnson *et al.*⁸⁾ isolated heterotrophic bacteria belonging to genera of *Pseudomonas*, *Achromobacter*, *Vibrio*, *Flavobacterium*, *Hyphomicrobium*, *Bacillus* and *Brevibacterium* from bottom muds of the Indian Ocean. Wood⁷⁾ also isolated *Staphylococcus*, *Sarcina*, *Corynebacterium* and *Mycoplana* from marine muds of eastern Australia. Bacterial members belonging to the genera *Pseudomonas* and *Aeromonas* were isolated from deep-sea sediments collected at depths from 9,400 to 10,400 m in the Philippine and Marianas Trenches of the Pacific Ocean.⁹⁾ On the other hand, Hayashi¹⁰⁾ studied heterotrophic bacterial flora in the bottom sediments of the Kumano Nada in Japan. According to him,¹⁰⁾ *Bacillus* (about 30–75%) was the most dominant bacterial genus. The unknown bacterial group which is gram positive, asporogenous motile rod with peritrichous flagella was also abundant (about 16–29% of the total heterotrophic isolates).¹⁰⁾ Gram positive bacteria including *Bacillus*, *Corynebacterium*, *Micrococcus* and the unknown bacteria comprised about 68–97% of total isolates from bottom sediments of the Kumano Nada (480–3,470 m in depth.)¹⁰⁾ The generic composition of lytic enzyme-producing bacteria is almost similar to that of sediment heterotrophic bacteria in the Kumano Nada.

Gram negative rods, such as *Pseudomonas*, *Vibrio*, *Alcaligenes*, *Flavobacterium* and *Acinetobacter-Moraxella* are generally dominant heterotrophic bacterial members in the water,¹⁰⁾ although gram positive microorganisms, such as *Bacillus*, *Micrococcus* and coryneforms are abund-

Table 4. Generic composition of the lytic enzyme-producing bacteria isolated from bottom sediments of coastal and oceanic regions of Japan

	Ise, Matoya and Owase Bays	Kumano Nada area	Nansei Shotō area	Total
Number of strains tested	328	80	74	482 (100.0%)
<i>Bacillus</i>	304	76	41	421 (87.3%)
<i>Micrococcus</i>	8	0	0	8 (1.7%)
<i>Alcaligenes</i>	5	2	5	12 (2.5%)
<i>Moraxella</i>	0	0	4	4 (0.8%)
<i>Flavobacterium</i>	1	1	0	2 (0.4%)
<i>Pseudomonas</i>	1	0	0	1 (0.2%)
Unknown	9	1	24	34 (7.1%)

ant in the bottom sediments.⁹⁾

Nair *et al.*¹⁾ studied bacteriolytic bacteria isolated from seawater, particulate materials and plankton of Tokyo, Sagami and Suruga Bays. The major bacteriolytic group was *Pseudomonas* at Sagami and Suruga Bays, whereas it was *Vibrio* at Tokyo Bay.¹⁾ However, in this study, gram negative rods, such as *Alcaligenes*, *Moraxella*, *Flavobacterium* and *Pseudomonas* were minor components of lytic enzyme-producing bacteria in the bottom sediments.

Nair *et al.*¹⁾ also indicated that about 24–52% of the bacterial isolates obtained from seawater of Tokyo, Sagami and Suruga Bays of Japan were capable of lysing *Vibrio parahaemolyticus* cells. More than 60% of the isolates from zooplankton produced lytic enzymes.¹⁾ In this study, the average percentage of bacteriolytic strains in marine sediments was 12.1%. The differences in percentage of bacteriolytic strains may be due to the differences in the substrate cells used for detecting lytic activity.

The temperature of water exceeding 1,000 m in depth is below 5°C in coastal and pelagic regions.¹¹⁾ About 19.1% (92 strains) of the bacteriolytic isolates (482 strains) from bottom sediments were able to grow at 5°C. This value (19.1%) seems to be rather low, probably due to the incubation temperature (25–30°C) used when bacteriolytic bacteria were firstly isolated from marine sediments.

Most of bacteriolytic isolates from marine sediments were *Bacillus* sp. It is still unknown, however, whether *Bacillus* species exist in the state

of the vegetative cells or spore. A significance of lytic enzymes and their role in marine environment are also not yet clear. Further study on the lytic enzyme-producing bacteria is necessary from the viewpoint of marine ecology.

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