Distribution and Activity of Heterotrophic Bacteria in the Mudflat of Nakdong River Estuary

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洛東江 河口 干潟地에 存在하는 細菌의 分布및 生理的 活性度

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Distribution pattern and activity of heterotrophic bacteria were measured in the mudflat of Nakdong river estuary. In March and June, 1985, community sizes of amylolytic, lipolytic and proteolytic bacteria as well as total viable counts were measured. Vertical distribution of bacterial community size increased a few orders of magnitude from March to June. Heterotrophic activity was estimated in turnover time with U-[^{14}C]-glucose. Turnover time reduced considerably in June compared to that of March. To examine correlations for measured bacterial groups, turnover time and environmental factors, correlation coefficient matrix was obtained. These measured characteristics did not consistently correlate well with one another.

Microbial communities associated with sediments are important to the decomposition of organic matter (Johnson and Calder, 1973; Litchfield, 1973), to certain geochemical transformations (Ehrlich, 1963; Temple, 1964; Pearcy *et al.*, 1977), to nutrient regeneration (Sugahara *et al.*, 1974; Wright, 1974; Aller and Yingst, 1980) and as food sources for benthic fauna (Gieskes and Kray, 1977; Skjoldal and Lännergren, 1978; Smetacek, 1980; Wassman, 1983).

Heterotrophic activity of bacteria in the aquatic and sedimental environments plays an important role in the regeneration of inorganic nutrients via the conversion of organic compounds to carbon dioxide, ammonia and hydrogen sulfide (Litchfield *et al.*, 1979; Griffiths *et al.*, 1981). This activity also result in the conversion of dissolved substrates into particular matter through increased biomass, which, in turn, can enter the food webs. Bacterial activity (turnover time) suggest that detritus plays an important role as a source of organic nutrients in inshore aquatic ecosystems. Approximately 50% of primary production in marine systems ends up as detritus (Fenchel and Jorgensen, 1977). Heterotrophic bacteria play a vital role in making that material available to higher trophic levels by conversion of soluble organic compounds to particulate carbon, hydrolization of recalcitrant compounds and enriching the detritus with organic nitrogen and phosphate. Therefore measurement of total viable counts, amylolytic, lipolytic and proteolytic bacterial

groups and turnover time of specific substrate contributes to the understanding of remineralization process as a essential role of bacteria in the mudflat ecosystem.

MATERIALS AND METHODS

Research areas and sampling procedures

Mudflat samples were collected with the aid of a PVC sediment corer (10 cm diameter, 50 cm length) from Nakdong River Estuary in the season of 30 March, 1985 and 1 June, 1985. Precise descriptions of research areas and sampling procedures were previously reported (Hong et al., 1985).

Enumeration

Total viable count; Marine Agar 2216e medium (Oppenheimer and ZoBell, 1952) with the distilled water was used for the total viable counts. Serial dilutions of the homogenized mudflat samples were poured into the agar medium. The inoculated plates were incubated for 7 days at 25°C. Developed colonies were counted with the aid of a Harris colony counter CC 30 (Philip Harris Ltd. Shenstone, England).

Amylolytic, lipolytic and proteolytic bacteria; For the evaluation of amylolytic, lipolytic and proteolytic bacteria, the sole carbon and energy source of the meida were soluble starch, Tween 80 and gelatin respectively. The basal medium consisted of: NaCl 27.5g; $MgCl_2$ 5.0g; $MgSO_4$ 2.0g; $CaCl_2$ 0.5g; KCl 1.0g; $FeSO_4$ 0.001g; Agar 15.0g; 1000ml distilled water (Pelczar, 1957). Soluble starch, Tween 80 and gelatin were added to the basal medium to 0.2%, 1.0% and 0.4% as final concentration. Number of amylolytic bacteria was counted out after addition of Gram's iodine solution. Developed colonies in Tween 80 medium with the zones of opacity were counted as those of lipolytic bacteria. Colonies having the more opaque zones than gelatin medium on flooding with 1% aqueous tannic acid were obtained as proteolytic bacteria (Holding and Collee, 1971).

Turnover time

Each diluents after pre-filtration with 62 μm pore size sieve were distributed into 3 parallels of 250ml sterile Erlenmeyer flask up to 50ml. U-[^{14}C]-glucose (Amersham International plc, Amersham, UK) was added so that the final concentration of labelled glucose was 1.0μg/l. A blank sample was fixed by 0.2ml of formalin filtered with 0.45μm pore size membrane filter. The samples were then incubated for 1.5 hours in the dark at *in situ* temperature with shaking water bath. After incubation, 0.2ml of formalin was added to samples for inhibitin of glucose uptake. Fixed samples filtered through 0.2 μm millipore filter and filtered membrane added to scintillation vial with 5ml of Dioxane cocktail solution. Average cpm values were counted for 2 min with the aid of a Packard Tri-Carb Liquid Scintillation Spectrometer model 3385 (Packard Co., USA). Turnover time (Tt) was calculated as follows;

Table 1. *Seasonal and vertical distribution of total viable counts(x10^7 colony-forming units/g-dry weight)*

Depth	Site S		Site M	
(cm)	March	June	March	June
1	3.9	13.0	2.8	24.0
2	4.1	14.0	1.7	12.0
3	5.7	19.0	9.5	5.3
4	3.9	N.D.	N.D.	4.9
5	3.9	12.0	14.0	N.D.
6	3.7	170.0	9.1	13.0
7	2.5	80.0	9.5	N.D.
8	3.5	N.D.	6.7	7.5
9	4.1	33.0	6.0	14.0
10	5.0	38.0	8.9	8.3
11	5.2	47.0	7.5	8.8
13	7.3	N.D.	9.5	6.3
15	5.2	N.D.	7.1	N.D.
17	3.3	N.D.	8.8	23.0
19	6.8	55.0	6.4	6.3
21	4.3	32.0	6.4	6.9
23	4.2	32.0	11.0	9.7
25	8.1	N.D.	6.3	7.4
27	2.5	N.D.	9.2	13.0
29	30.0	N.D.	6.7	N.D.

N.D. : not determined

$$Tt = t \times \frac{1}{f}$$

t; incubation time (hours)

f; $\frac{\text{net uptake cpm}}{\text{standard cpm}}$

RESULTS

Table 1 and 2 showed seasonal and vertical distributions of total viable counts, amylolytic, lipolytic and proteolytic bacteria in the site M and the site S. In March at the site S total viable counts was 5.9×10^7 colony-forming units (cfu)/g-dry weight (g-d.w.) as mean value. Average values from whole depths of amylolytic, lipolytic and proteolytic groups were 1.5×10^6 cfu/g-d.w., 2.0×10^6 cfu/g-d.w. and 2.2×10^6 cfu/g-d.w., respectively. Generally lipolytic and proteolytic bacterial numbers were higher than that of amylolytic bacteria. In March at the site M mean value of total viable counts was measured to 7.7×10^7cfu/g-d.w.. Number of amylolytic, lipolytic and proteolytic bacteria was more or less higher than that in March at the site S and ratios of bacterial groups to total viable counts were slightly higher than those at the site S.

In March maximal ratios of amylolytic, lipolytic and proteolytic bacteria to total viable counts of site S were observed in the depth of 6cm-8cm layer, but those values were relatively low at the range of 5.2% to 10.4%. Those of site M were showed in surface layer to 2cm layer for 5.4% to 24.7%.

Table 2. *Seasonal and vertical distribution of amylolytic, lipolytic and proteolytic bacteria ($\times 10^6$ colony-forming units/ g-dry weight)*

Depth (cm)	Site S						Site M					
	March			June			March			June		
	Am. [a]	Li [b]	Pr. [c]	Am.	Li.	Pr.	Am.	Li.	Pr.	Am.	Li.	Pr.
1	–	2.8	2.2	51.0	8.2	29.0	2.4	3.9	3.0	–	7.9	160.0
2	1.1	2.1	1.6	36.0	4.4	580.0	0.9	4.2	2.2	12.0	5.9	76.0
3	1.8	1.7	2.3	–	8.3	67.0	3.0	3.3	2.8	0.9	9.2	37.0
4	1.8	3.0	2.7	–	110.0	140.0	2.7	6.0	3.2	17.0	9.8	37.0
5	1.6	1.9	2.3	130.0	7.8	67.0	6.9	5.5	5.6	0.7	6.0	29.0
6	1.1	1.5	2.9	260.0	9.6	62.0	3.7	2.2	5.0	28.0	7.5	40.0
7	1.3	2.6	1.9	41.0	12.0	140.0	4.8	6.0	6.5	14.0	12.0	–
8	2.0	3.2	1.6	32.0	9.1	34.0	1.9	3.3	4.2	21.0	13.0	50.0
9	–	2.8	2.0	33.0	–	56.0	2.5	–	2.4	–	7.6	76.0
10	1.6	1.2	3.0	33.0	7.5	50.0	4.8	3.7	3.2	0.7	19.0	33.0
11	1.5	3.7	1.9	33.0	11.0	39.0	2.0	5.4	3.2	10.0	13.0	53.0
13	2.0	3.2	1.9	38.0	24.0	130.0	2.6	3.6	2.5	13.0	–	44.0
15	1.1	2.2	1.6	28.0	36.0	–	1.1	2.9	2.0	15.0	8.1	97.0
17	–	1.7	1.8	–	14.0	95.0	1.4	3.7	3.5	28.0	17.0	20.0
19	1.3	1.2	1.3	210.0	–	120.0	1.4	1.7	1.7	31.0	4.7	75.0
21	0.9	1.6	1.9	230.0	–	110.0	1.3	4.8	2.3	160.0	5.6	250.0
23	1.5	1.3	0.9	–	76.0	120.0	2.8	4.1	3.1	35.0	8.1	65.0
25	1.4	3.7	1.5	46.0	5.4	46.0	1.6	3.1	1.4	120.0	13.0	94.0
27	1.0	1.3	1.6	53.0	56.0	–	2.6	3.4	3.8	–	6.5	45.0
29	1.9	2.1	2.2	160.0	88.0	320.0	1.7	2.5	1.7	35.0	7.4	76.0

a; Amylolytic bacteria b; Lipolytic bacteria c; Proteolytic bacteria

In June total viable counts and the number of amylolytic, lipolytic and proteolytic bacterial groups were rapidly increased at the level of one or two order of magnitude contrasted with March. Also significant increase of proportions of bacterial groups to total viable counts was observed in both sites, especially the ratio of proteolytic bacterial group was measured to 56.8% in the site S and 82.2% in the site M. Whereas the number of lipolytic bacteria was increased more or less, the ratio of lipolytic bacteria was slightly increased in the site M for 11.1% or decreased in the site S for 3.3%. The ratio of physiological groups of bacteria in June was fluctuated very much from the depth of mudflat samples and its value was much higher than that of March.

Measuring of turnover time was performed at *in situ* temperature. In March of the site M, turnover times were above 393 hours and its maximal time was 10769 hours in depth of 21 cm. At the site S of the same period, turnover time was relatively shorter (102-4142 hours) than that of the site M as shown in Table 3. In June turnover time of both sites considerably decreased compared to those of March, 49-352 hours of the site M and 65-191 hours of the site S.

Table 3. *Turnover time measured with U-[^{14}C]-glucose in March and June (hour)*

Depth (cm)	Site S		Site M	
	March	June	March	June
1	125	88	422	151
2	153	93	393	50
3	102	76	1826	53
4	323	102	2197	89
5	649	109	1584	91
6	1561	90	539	57
7	3474	89	713	58
8	–	94	567	74
9	2692	178	2627	111
10	1210	93	758	143
11	–	65	1134	49
13	682	90	962	108
15	4142	168	1561	115
17	905	65	2761	102
19	1016	143	1825	203
21	595	148	10769	328
23	2761	180	5982	352
25	1398	191	1538	139
27	2991	186	–	417
29	1313	92	2243	284

Correlation coefficients between the bacterial groups, turnover time and environmental factors were shown in Table 4. In June significant negative correlation existed between turnover time and organic matter, total nitrogen in both research sites. In March at the site S negative correlation was found only between turnover time and organic matter. Other meaningful correlations were not observed.

DISCUSSIONS

Total viable counts and amylolytic, lipolytic and proteolytic bacteria were rapidly increased from March to June in both study sites. Heterotrophic activity (turnover time) was also shortened in the same period. These might be influenced with the change of temperature, which was 6°C-8°C in March and 21°C in June (Hong *et al.*, 1985) and the concentration of total nitrogen and inorganic phosphate.

Turnover time from March to June reduced 0.07 times at the site M and to 0.08 times at the site S whereas total viable counts were increased for 1.4 times and 7.9 times at each sampling sites respectively. Wright and Habbie (1966) found a value of Q_{10} for the maximal uptake velocity of 2.2. And an increase in temperature of about 15°C might lead a rise of V_{max} by about a factor of 3-4 (Gocke, 1977). Heterotrophic activity measured by turnover time in the research area proposed a good suggestion for bacterial activity. Turnover time also suggest the evidence that detritus plays an important role as a source of organic nutrients in inshore ecosystems.

When discussing the major role of bacteria in energy flow and substrate transfer at sedimental ecosystem, bacteria were regarded to exploit dead organic material as a major source of energy and carbon. From this aspect bacteria have several properties as microconsumer in ecosystems; utili-

Table 4. *Correlation coefficient matrix for several bacterial groups, turnover time and environmental factors.*

March

Site S / Site M	Sa. [a]	Am. [b]	Pr. [c]	Li. [d]	Tt. [e]	OM. [f]	TN. [g]	Ph. [h]
Sa.		0.369	0.066	0.031	−0.088	−0.217	−0.272	−0.309
Am.	0.708		0.205	0.409	−0.398	0.209	0.172	0.302
Pr.	0.554	0.747		−0.042	−0.357	0.550	0.502	0.465
Li.	0.238	0.401	0.473		−0.070	0.018	0.248	0.105
Tt.	0.089	−0.221	−0.229	0.161		−0.430	−0.232	−0.420
OM.	−0.141	0.242	0.121	0.209	−0.300		0.854	0.406
TN.	−0.326	0.158	−0.046	0.261	−0.285	0.899		0.413
Ph.	−0.479	−0.053	−0.103	0.095	−0.304	0.726	0.789	

June

Site S / Site M	Sa.	Am.	Pr.	Li.	Tt	OM.	TN.	Ph.
Sa.		0.522	−0.188	−0.064	−0.156	−0.276	0.272	0.307
Am.	−0.101		−0.013	0.217	−0.014	−0.139	0.042	0.050
Pr.	0.075	0.843		0.201	−0.118	0.135	−0.197	−0.044
Li.	0.093	−0.172	−0.381		0.300	−0.215	−0.387	−0.330
Tt	0.000	0.556	0.377	−0.342		−0.603	−0.508	−0.657
OM.	−0.021	−0.494	−0.201	0.055	−0.509		0.704	0.555
TN.	0.080	−0.447	−0.051	−0.220	−0.569	0.840		0.839
Ph.	0.273	−0.187	−0.023	−0.411	−0.065	−0.166	0.048	

a; Saprophytes b; Amylolytic bacteria c; Proteolytic bacteria
d; Lipolytic bacteria e; Turnover time f; Organic matter
g; Total nitrogen h; Inorganic phosphate

zation of dissolved organic substrates at the low concentrations, assimilation of dissolved inorganic nutrients such as nitrate and phosphate, decomposition of nutrient-poor plant tissue, hydrolyzation of structural plant compounds efficiently and ability of efficient metabolic system in anoxic state (Fenchel and Jorgensen, 1977). In the oceans and lakes dissolved organic matter, particulate organic matter and living biomass are found in proportions of *ca.* 100:10:2 (Parsons, 1963; Wetzel *et al.*, 1972). Low concentrations of organic matter were mainly used as energy and carbon source for bacterial secondary production which resulted in introduction to higher trophic levels. Therefore the evaluation of colony-forming units and turnover time was regarded as fundamental work and vertical distribution of bacteria and turnover time for specific substrate suggested basic data for understanding of energy and substrates flow in detritus food chain.

Litchfield *et al.* (1975) observed from marine sediment in Raritan Bay that bacterial number in surface laver (0-5cm) of 7 orders of magnitude decreased to 4-5 orders (*ca.* below 15cm). Bacterial distribution in a sediment core from southern Persian Gulf reduced rapidly beneath 5cm depth (Rheinheimer and Gunkel, 1974). Biomass from ATP measurement showed the similar results (Christian and Wiebe, 1978; Aller and Yingst, 1980). But distribution of bacteria in research area was relatively uniform in the range of 10^7 – 10^8 cfu/g-d.w.

Highly significant negative correlation was between bacterial numbers from direct counts and

Table 5. *Seasonal and vertical contents of organic matter, inorganic phosphate and total nitrogen.* *

Depth (cm)	Site S						Site M					
	March			June			March			June		
	OM. [a]	Ph. [b]	TN. [c]	OM.	Ph.	TN.	OM.	Ph.	TN.	OM.	Ph.	TN.
1	1.84	136.2	0.15	1.72	116.6	0.45	2.29	146.0	0.40	2.72	130.2	0.65
2	1.92	126.4	0.20	1.87	115.5	0.45	2.07	116.6	0.35	1.91	136.2	0.55
3	2.31	131.2	0.30	1.92	100.8	0.40	2.16	123.9	0.35	2.14	138.6	0.45
4	2.51	143.5	0.50	1.78	111.7	0.45	2.06	104.3	0.40	3.38	123.9	0.55
5	3.13	138.6	0.45	1.69	123.9	0.60	2.08	69.7	0.25	3.01	143.5	0.65
6	2.28	114.1	0.35	1.51	128.8	0.55	1.63	86.8	0.15	1.93	131.3	0.45
7	1.72	97.0	0.30	1.60	128.8	0.50	1.30	67.2	0.10	1.95	89.6	0.45
8	1.79	136.2	0.20	1.66	128.8	0.60	1.22	89.6	0.10	3.48	86.8	0.60
9	1.72	123.9	0.20	1.29	121.5	0.45	1.41	97.0	0.20	4.09	74.6	0.70
10	1.52	143.5	0.10	1.61	128.8	0.55	1.25	77.0	0.25	2.63	62.3	0.40
11	1.71	121.5	0.35	1.81	133.7	0.50	1.25	97.0	0.15	1.96	94.5	0.30
13	1.13	138.6	0.15	1.42	119.0	0.45	1.30	84.4	0.10	3.00	69.7	0.50
15	1.02	131.3	0.15	1.08	106.8	0.30	1.42	67.2	0.15	2.55	74.6	0.45
17	1.50	128.8	0.15	1.10	101.9	0.30	1.27	42.7	0.10	2.08	101.9	0.35
19	1.25	119.0	0.10	1.12	101.9	0.35	1.21	64.8	0.10	2.67	64.8	0.70
21	1.35	121.5	0.15	1.53	116.6	0.45	1.16	67.2	0.10	1.56	104.3	0.30
23	1.32	74.6	0.10	1.39	75.6	0.35	1.30	74.6	0.10	1.09	111.7	0.15
25	1.26	64.8	0.10	1.11	55.0	0.25	1.27	62.3	0.10	1.29	64.8	0.25
27	1.29	57.4	0.10	1.16	64.8	0.25	-	-	-	1.28	104.3	0.15
29	1.39	79.5	0.10	1.26	101.9	0.30	-	-	-	1.49	114.1	0.30

a; Organic matter (w/w percentage) b; Inorganic phosphate (μg/g-dry weight)
c; Total nitrogen (mg/g-dry weight) *; courtesy of Joon Ho Kim (personal communication)

mean grain size, this suggested that the surface area of the grains was important for the development of a dense bacterial population. Significant positive correlation was in bacterial numbers with sedimental organic carbon and organic nitrogen content and those contents negatively correlated to the grain size (Dale, 1974).

In sediment surface bacterial population was under the influence of ecological situation. Phytoplankton blooming occured at spring and autumn and organic materials accumulated in the sediment at winter season (Meyer-Reil, 1983).

Communities of *Phragmites communis* were well developed in research area, these might be one of the important nutrient sources input to sedimental ecosystem. In research area organic matter, total nitrogen and inorganic phosphate were more or less homogeneous (Table 5). As previously described, research area appeared to atmosphere on the ebb tide (Hong *et al.*, 1985) and effects of the flooding of Nakdong river and tide resulted in topographical changes and erosion of mudflat surface. These might be the causes of distribution of bacteria and nutrients. Unfortunately significant correlations between bacterial numbers and nutriens were not found in research area, these could be due to evaluate the numbers of aerobic heterotrophs instead of direct counts of bacteria and to result from masking effects of difference in bacterial population.

Whereas significant correlation between total viable counts and turnover time was not observed in research area at March and June, large increase of aerobic heterotrophs corresponds somewhat to decrease of turnover time. And aerobic heterotrophs may contribute greatly to regeneration of

nutrients not only in surface of mudflat but also lower layer. To comprehed the function of mudflat ecosystem more precisely, the relationship and interaction between aerobic and anaerobic bacterial community have to be studied.

摘 要

洛東江 河口 流域의 干潟地에 存在하는 細菌의 分布 狀況 및 그 生理的 活性度를 調査하였다. 30cm 깊이도 採集한 堆積土壤標本에서 總細菌數, 澱粉分解細菌數, 脂肪分解細菌數, 蛋白質分解細菌數를 測定하였으며, 1985年 3月에 比하여 6月에 曾加하는 樣相을 나타내었으며, 깊이別에 따른 細菌수의 変化를 觀察하였다. U-[^{14}C]-glucose의 利用度에 依하여 生理的 活性度를 測定하였다. 細菌群集과 Turnover time 및 環境要因들 사이의 相關度를 計算하였다. 이러한 測定値 사이에서 지속적인 相關關係를 發見하지 못하였다.

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