Characteristics of Heterotrophic Bacteria and Their Relationships with Environmental Parameters in Naktong Estuary

Kwon, O.S. and *Y.C. Hah

Dept. of Environmental Science, Inje College
**Dept. of Microbiology, Seoul National University*

낙동강 하구 생태계의 종속영양세균의 특성 및 환경요인과의 관계

권오섭·하영칠*

인제대학 환경학과, *서울대학교 미생물학과

ABSTRACT: Samples from Naktong Estuary had been taken for the characterization of heterotrophic bacterial communities and of the effects of environmental factors on their distribution in estuarine ecosystem. Bacterial communities isolated from seawater region were composed of more euryhaline groups than those from freshwater region, and the bacterial communities of summer were composed of more eurythermal groups than those of winter. Bacterial communities became more diverse by the input of allochthonous bacteria from terrestrial and freshwater ecosystem, but less diverse by worse environmental conditions such as nutrient load, high salinity, low temperature, and so on.

KEY WORDS□diversity index, evenness index, physiological tolerance index, heterotrophic bacteria, Naktong Estuary.

There have been several trials to describe the heterotrophic bacterial community structure in natural habitats by viable countings of bacterial number followed by their isolation and grouping. Among various methods, here we characterized bacterial community by using diversity index. Diversity indices frequently are used to describe community structure for higher organisms, but relatively few papers concerning bacteria have used such indices because the definition of species for bacteria is difficult (Mandel, 1969). Although, the concept of a species diversity is difficult to define for bacteria and probably is not applicable to bacteria in the true sense, it is possible to utilize the same calculations to describe taxonomic diversity within the bacterial community utilizing arbitrarily defined phenotypic clusters, generated from numerical taxonomic analyses and considered functionally equivalent to species (Sokal and Sneath, 1963; Kaneko *et al.*, 1977).

Estuaries may receive nutrients through upwelling of deeper water masses along the continental shelf, but the larger portion of nutrient input usually comes from the adjacent land in the form of runoff. Thus, estuarine ecology should be envisioned as a sector that is highly dynamic in all dimensions (Zobell, 1973). We investigated, therefore, the diversity indices of heterotrophic bacterial communities and their relationships with environmental parameters in Naktong Estuary.

MATERIALS AND METHODS

Collection of Samples

Four stations located in Naktong Estuary were sampled between July 1985 and December 1986 once a month (Fig. 1). Samples were collected about 1 meter under the water surface at St. 1,2,

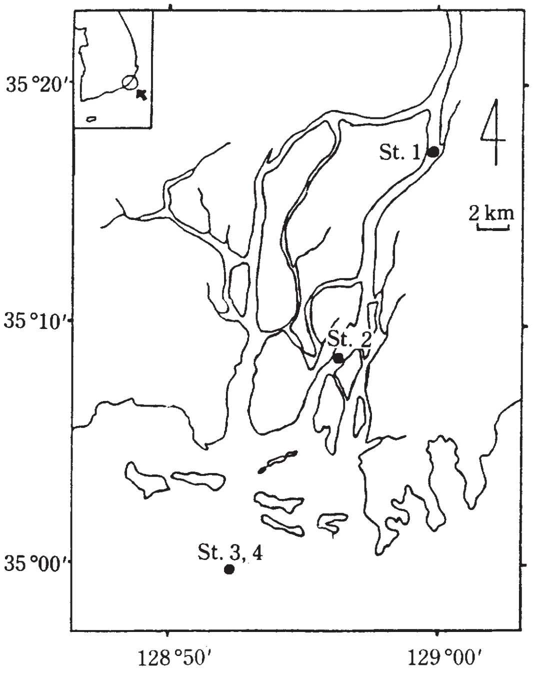

Fig. 1. *Map showing the sampling stations in Naktong Estuary*

and 3, and about 20 meters under the surface at St. 4 using a Van-dorn sampler which was thoroughly washed with water taken from each station before sampling. Thirteen parameters of water quality (temperature, salinity, pH, dissolved oxygen, biochemical oxygen demand (BOD), modified BOD (MBOD), MBOD-N, MBOD-P, ammonia, nitrite, nitrate, phosphate, and chlorophyll a), and six bacteriological parameters (heterotrophic, lipolytic, casinolytic, spore-forming, total coliform, and fecal coliform bacteria) were determined. The data of precipitation and radiation were quoted from Monthly Weather Report (Anon., 1985 and 1986). More detailed descriptions of station and methods of parameters were mentioned in Ahn (1988) and Kwon (1988).

Characterization and Clustering of Bacterial Isolates

Procedures for determining bacterial counts and the isolation of predominant bacterial strains have been described in a previous paper (Kwon *et al.*, 1987). A total of 72 morphological and physiological characters were examined for each isolates, and the methods for each cluster were also fully described in previous reports (Kwon *et al.*, 1987; Kwon, 1988).

Data Analysis

The calculation of similarity index (S value) was carried out according to the following equation: $S(\%) = Ns \times 100/(Ns + Nd)$, where Ns represents the number of characters shared by two strains, and Nd the number of characters differing from each other. Cluster analysis was carried out according to the clustering method of single linkage clustering (Sokal and Sneath, 1963).

The number of taxonomic groups and of individuals within each clusters, determined by the cluster analysis, were used for calculation of Shannon diversity index, H′(Pielou, 1966). H′ is defined by the following equation: $H' = -P_i \, Log(P_i)$, where P_i represents the number of individuals in a cluster divided by the total number of isolates in the sample analyzed.

Evenness index, J′(Pielou, 1975), was calculated according to the following equation: $J' = H'/H_{max}$, where H_{max} is the maximal value of H′ for a given sample size.

Physiological tolerance indices were used to describe the capacity of the bacterial community to tolerate deviations from ambient conditions of temperature, salinity, and pH (Hauxhurst *et al.*, 1981). The basic formula used to calculate the tolerance index is as follows: $Px = Gx_i/n$, where Px is the tolerance index for x, Gx_i represents the proportion of the population capable of growth at the specified condition of parameter x, and n represents the number of specific condition of x examined.

Pearson correlation coefficients were calculated between the measured parameters.

RESULTS AND DISCUSSION

A total of 1044 strains were isolated, and grouped by cluster analysis (Table 1). Bianchi and Bianchi (1982) have found that 20-30 strains were sufficient to evaluate a diversity of bacterial community without any influence on bacterial diversity. But diversity index is sensitively changed in cases of small number of isolates (Mills and Was-

Table 1. *Number of isolated bacteria and clusters (in parenthesis) in Naktong Estuary.*

	St.1	St.2	St.3	St.4	sum
85. 7.26.	19(7)	19(9)	18(11)	13(9)	69
8.15.	22(14)	17(10)	16(12)	13(8)	68
9.27.	18(12)	15(9)	17(8)	22(5)	72
10.27.	5(4)	7(5)	16(7)	10(5)	38
12. 1.	18(9)	19(6)	22(10)	17(8)	76
12.27.	6(3)	11(4)	18(8)	13(7)	48
86. 1.23.	8(5)	12(6)	22(12)	19(7)	61
2.24.	9(5)	6(3)	16(5)	6(5)	37
3.27.	14(5)	13(5)	23(4)	19(5)	69
4.21.	22(10)	19(8)	19(7)	14(8)	74
5.25.	22(8)	32(12)	23(8)	30(10)	107
6.27.	25(9)	27(13)	36(12)	31(16)	119
8. 5.	30(12)	26(7)	26(11)	23(10)	105
10.31.	13(8)	29(9)	29(10)	30(8)	101
sum	231	252	301	260	1044

sel, 1980), the numbers of isolates in this study were sometimes not satisfied with the suggestion of Bianchi and Bianchi (1982).

Not only H′ but also J′ are presented in Table 2. Mean H′ value of each station varied from 2.49 to 2.80, and mean J′ value from 0.62 to 0.67. A J′ value of 1 shows an even distribution; when J′ is near 0 there is uneven distribution of individuals within the taxa of the community. Thus it could be known that, although the numbers of isolates were sometimes insufficient and some differences of H′ value (1-2) existed in the samples from September to December 1985, there was no significant difference in mean taxonomic diversities among stations. Diversity indices of winter months were lower than those of other seasons, which means that the bacterial community in winter is less diverse than the other seasons, probably due to the physical stress. Diversity was relatively lower in Naktong Estuary than in oligotrophic marine ecosystems(Martin and Bianchi, 1980; Hauxhurst *et al.*, 1981; Bianchi and Bianchi, 1982; Horowitz *et al.*, 1983).

The physiological tolerance indices for growth over the ranges of temperature (Pt), pH (Ph), and salinity (Ps) are shown in Table 3. Considerable variations in Pt were apparent at different months, although there was no significant difference between mean value of Pt at each station. The higher values were found in summer and lower values in winter. Thus, the bacterial communities in summer are supposed to be more tolerant to chan-

Table 2. *Shannon's diversity index (H') and evenness index (J') of heterotrophic bacterial communities in Naktong Estuary*

	St. 1		St. 2		St. 3		St. 4		mean	
	H′	J′	H′	J′	H′	J′	H′	J′	H′	J′
85. 7.26.	2.48	0.58	2.75	0.65	3.19	0.77	3.03	0.82	2.87	0.71
8.15.	3.55	0.80	3.15	0.77	3.50	0.88	2.87	0.78	3.27	0.81
9.27.	3.39	0.81	2.87	0.73	2.77	0.68	1.21	0.27	2.56	0.62
10.27.	1.92	0.83	2.13	0.76	2.73	0.68	2.25	0.68	2.26	0.74
12. 1.	2.82	0.68	2.19	0.52	3.02	0.68	2.27	0.56	2.58	0.61
12.27.	1.46	0.56	1.79	0.52	2.59	0.62	2.57	0.69	2.10	0.60
86. 1.23.	2.16	0.72	2.46	0.69	3.45	0.77	2.55	0.60	2.66	0.70
2.24.	2.06	0.65	1.79	0.69	2.11	0.53	1.46	0.56	1.86	0.61
3.27.	1.81	0.48	2.03	0.55	1.80	0.40	1.93	0.45	1.89	0.47
4.21.	2.95	0.66	2.78	0.65	2.27	0.53	2.50	0.66	2.63	0.63
5.25.	2.64	0.59	2.99	0.60	2.49	0.55	3.05	0.62	2.79	0.59
6.27.	2.84	0.61	3.41	0.72	3.21	0.62	3.77	0.76	3.31	0.68
8. 5.	3.14	0.64	2.15	0.46	3.04	0.65	3.06	0.68	2.85	0.61
10.31.	2.87	0.78	2.33	0.48	3.09	0.64	2.51	0.51	2.70	0.60
mean	2.58	0.67	2.49	0.63	2.80	0.64	2.50	0.62	2.59	0.64

Table 3. *Physiological tolerance indices for temperature (Pt), pH(Ph), and salinity (Ps) of bacterial communities isolated from each station of Naktong Estuary.*

	St. 1			St. 2			St. 3			St. 4		
	Pt	Ph	Ps	Pt	Ph	Ps	Pt	Ph	Ps	Pt	Ph	Pt
85. 7.26.	0.69	0.56	0.61	0.50	0.42	0.59	0.58	0.33	0.62	0.89	0.58	0.57
8.15.	0.83	0.68	0.62	0.74	0.59	0.52	0.77	0.60	0.66	0.54	0.46	0.65
9.27.	0.72	0.74	0.66	0.48	0.57	0.57	0.33	0.47	0.55	0.79	0.98	0.79
10.27.	0.80	0.60	0.40	0.38	0.22	0.17	0.63	0.60	0.76	0.63	0.55	0.68
12. 1.	0.22	0.34	0.29	0.35	0.24	0.19	0.15	0.52	0.37	0.43	0.50	0.41
12.27.	0.44	0.67	0.53	0.24	0.50	0.47	0.54	0.50	0.77	0.46	0.69	0.82
86. 1.23.	0.13	0.07	0.35	0.50	0.29	0.53	0.45	0.50	0.68	0.33	0.50	0.64
2.24.	0.37	0.50	0.89	0.34	0.67	0.43	0.33	0.82	0.78	0.22	0.50	0.47
3.27.	0.15	0.22	0.27	0.21	0.25	0.31	0.16	0.52	0.61	0.14	0.58	0.59
4.21.	0.21	0.35	0.48	0.28	0.37	0.67	0.23	0.53	0.48	0.33	0.68	0.72
5.25.	0.55	0.57	0.46	0.59	0.44	0.64	0.43	0.57	0.57	0.67	0.72	0.81
6.27.	0.44	0.26	0.49	0.75	0.50	0.56	0.71	0.68	0.74	0.76	0.57	0.82
8. 5.	0.65	0.50	0.41	0.68	0.33	0.55	0.74	0.41	0.74	0.65	0.48	0.83
10.31	0.51	0.56	0.55	0.67	0.80	0.66	0.64	0.38	0.66	0.48	0.45	0.65
mean	0.48	0.47	0.50	0.48	0.44	0.49	0.48	0.53	0.64	0.52	0.59	0.68

ges in temperature, that is, composed of eurythermal groups. This suggests that the bacterial communities of summer are heterogenous and have high informational contents within the gene pool of the community, and they can thus survive throughout the year. In the variations of mean value of Ph and Ps, there was no significant differences between months, but differences existed between stations. As shown in Table 3, high values of tolerance indices for pH (Ph) and salinity (Ps) were observed at those stations where the heterotrophic bacteria were exposed to varied and higher levels of such parameters, suggesting that high pH and salinity support bacterial community with more versatility, whereas environment of low pH and salinity support with less versatility. The mean values of three tolerance indices were similar (Pt = 0.49, Ph = 0.51, Ps = 0 50). This indicates that the bacterial communities isolated from Naktong Estuary have similar tolerances to changes in temperature, pH, and salinity over the ranges tested.

In order to see the relationship between diversity and environmental parameters, Pearson's correlation coefficients were calculated (Table 4). Since water temperature in summer was ranged between 20° and 30 °C (Ahn, 1988), it was sufficient to grow for most psychrophilic and mesophilic bacteria and could amplify the bacterial activities of most species than at low temperature range, and made bacterial community more diverse. But diversity decreases markedly at too high temperature (Strom, 1985). Spore-forming bacteria and precipitation also positively correlated with diversity, since much input of soil bacteria by heavy rain could make the bacterial community more diverse. High concentration of dissolved oxygen could be said as the effects of low temperature and input of seawater, *i.e.*, high salinity in Naktong Estuary(Kwon, 1988). Low temperature, contrary to high temperature, could make the community less diverse by inhibiting the bacterial activities. High value of salinity also did the community less diverse, because most of bacterial types were originated from freshwater and terrestrial ecosystems of Naktong Estuary (Kwon, 1988) and could not survive in high salt environment. Such result also has been reported by Hunter *et al.* (1986) using a laboratory estuarine simulation experiment. Diversity had negative correlations with MBOD-P

Table 4. *Correlation coefficients of diversity index with environmental parameters in Naktong Estuary*

	TEM	SAL	DO	BOD	MBOD	M-N	M-P	NH_4	NO_2	NO_3
St. 1	.57		–*			–	–			
St. 2	+*	–	–.69					–.59	.63	
St. 3	+	–	–		–	–	–.68	–		+
St. 4		–		.62				–	–	+
**All	.42		–.36	+	–	–	–.36	–.44		

	LTP	CAS	N-0	N-10	Z-25	TC	FC	SPO	PRE
St. 1	+		–	–	–				
St. 2								.63	
St. 3						+	+	+	+
St. 4	+	.59	+	+		+	+	.54	+
**All								.36	.47

Statistical probability(p)<0.05, *p<0.2. **Correlation coefficient with all data of 4 stations.
TEM, water temperature; SAL, salinity; M-N, MBOD-N; M-P, MBOD-P; NO_2, nitrite; NO_3, nitrate; LIP, lipolytic bacteria; CAS, casinolytic bacteria; N-0, CFU on N-0 medium; N-10, CFU on N-10 medium; Z-25, CFU on Z-25 medium (Kwon *et al.*, 1987); TC, total coliform bacteria; FC, fecal coliform bacteria; SPO, spore-forming bacteria; PRE, precipitation

and ammonia, and the negative correlations were also found with MBOD and MBOD-N whose probability levels were, though, not significant ($p<0.2$). From these results it was known that nutrient loadings decreased diversity index. Wastewater is supposed to have effects on specific bacterial species, which can use or tolerate quickly these pollutants and make itself a dominant group in the ecosystem, thus the bacterial community becomes less diverse.

적 요

낙동강 하구 생태계의 종속영양세균의 특성과 이에 미치는 환경요인의 영향을 조사하였다. 해수역에 위치한 정점에서 분리한 세균군집이 담수역에서 분리한 군집보다 광염성 세균으로 이루어져 있으며, 하계에 분리한 군집이 동계군집에 비해 광온성세균으로 구성되어 있었다. 또한 수계의 세균군집은 육상에서 유입되는 비토착성세균에 의해 다양성이 커지며, 환경이 악화되면 세균군집의 다양성이 감소하는 현상이 관찰되었다.

ACKNOWLEDGEMENT

We deeply express sincere gratitude to the late Dr. S.W. Hong for his encouragement in the performance of this investigation. Thanks are also extended to Dr. T.S. Ahn, Dr. T.Y. Ahn, K.S. Joh, and D.Y. Jeon for assistance with various aspects of this work.

REFERENCES

1. Ahn, T.Y., 1988. Seasonal variation and salinity effect of microbial heterotrophic activity in the Naktong River Estuary. Ph.D. thesis. Seoul National Univ.
2. Anonymous, 1985 and 1986. Monthly weather report. Korea Meteological Service. Seoul. Korea.
3. Bianchi, M.A.G. and A.J.M. Bianchi, 1982. Statistical sampling of bacterial strains and its use in bacterial diversity measurement. *Microb. Ecol.* **8:** 61-69.
4. Hauxhurst, J.D., T. Kaneko, and R.M. Atlas, 1981. Characteristics of bacterial communi-

ties in the Gulf of Alaska. *Microb. Ecol.* **7:** 167-182.

5. Horowitz, A., M.I. Krichevsky, and R.M. Atlas, 1983. Characteristics and diversity of marine oligotrophic, stenoheterotrophic, and euryheterotrophic bacterial populations. *Can. J. Microbiol.* **29:** 527-535.
6. Hunter, M., T., Stephenson, P.W.W. Kirk, R. Perry, and J.N. Lester, 1986. Effect of salinity gradients and heterotrophic microbial activity on biodegradation of nitriloacetic acid in laboratory simulations of the estuarine environment. *Appl. Environ. Microbiol.* **51:** 919-925.
7. Kaneko, T., R.M. Atlas, and M. Krichevsky, 1977. Diversity of bacterial populations in the Beaufort Sea. *Nature.* **270:** 595-599.
8. Kwon, O.S., 1988. Analysis of environmental factors and characterization of heterotrophic bacterial communities in Naktong Estuary. Ph.D. thesis. Seoul National Univ.
9. Kwon, O.S., Y.C. Hah, and S.W. Hong, 1987. Variations of diversity and tolerance indices of heterotrophic bacterial communities in Naktong Estuary. *Kor. J. Microbiol.* **25:** 229-237.
10. Mandel, M., 1969. New approaches to bacterial taxonomy: perspectives and prospects. *Annu. Rev. Microbiol.* **23:** 239-274.
11. Martin, Y.P. and M.A. Bianchi, 1980. Structure, diversity, and catabolic potentialities of aerobic heterotrophic bacterial populations associated with continuous cultures of natural marine phytoplankton. *Microb. Ecol.* **5:** 265-279.
12. Mills, A.L. and R.A. Wassel, 1980. Aspects of diversity measurement for microbial communities. *Appl. Environ. Microbiol.* **40:** 578-586.
13. Pielou, E.C., 1966. Shannon's formular as a measure of species diversity: its use and misuse. *Am. Nat.* **100:** 463-465.
14. Pielou, E.C., 1975. Ecological diversity. John Wiley & Sons. New York.
15. Sokal, R.R. and P.H. Sneath, 1963. Principles of nemerical taxonomy. W.H.Freeman and Co. San Francisco.
16. Strom, P., 1985. Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. *Appl. Environ. Microbiol.* **50:** 899-905.
17. Zobell, C.E., 1973. Microbial and environmental transitions in estuaries. In: Estuarine microbial ecology, pp. 9-34. ed. by L.H. Stevenson and R.R. Colwell. Univ. South Carolina Press. Columbia.

(Received July 11, 1988)