KOR. JOUR. MICROBIOL. December, 1990 p. 331-336 Vol. 28, No. 4

Seasonal Distribution and Characteristics of Heterotrophic Marine Bacteria in the Intertidal Zone Near Kunsan of Yellow Sea, Korea

Lee, Geon-Hyoung, *Sang-Jin Kim, **Won-Ho Lee and Da-Mi Lee
*Department of Biology, **Department of Oceanography, Kunsan National University, Miryo ng-dong, San 68, Kunsan City, Jeon-Buk Do, 573-360, Korea*
**Genetic engineering Center, Korea Institute of Scinence and Technology, P.O. Box 17, Daeduk Science Town, Daejeon*

군산부근 조간대에서의 해양종속영양 세균의 계절적 분포와 특성

이건형 · 김상진* · 이원호** · 이다미
군산대학 생물학과
*한국과학기술연구원 유전공학센타
**군산대학 해양학과

ABSTRACT: Annual distribution of heterotrophic marine bacteria and seasonal characteristics were investigated in the intertidal waters and sediments in the vicinity of Kunsan of Yellow Sea, Korea. Annual distribution of heterotrophic marine bacteria ranged from 7.5×10^2 to 1.1×10^5 c.f.u./ml in water and from 1.6×10^4 to 4.8×10^6 c.f.u. per g dry sediments. As for the morphological distribution measured by epifluorescence microscopy, rod-form bacteria were distributed more than 74% of total observed bacteria during surveying periods. Average biovolume of sampled bacteria ranged from $3.19\pm0.59\times10^{-2}$ to $6.19\pm0.76\times10^{-2}$ μm^3 for coccus-form bacteria, and from $4.57\pm0.17\times10^{-2}$ to $12.94\pm0.21\times10^{-2}$ μm^3 for rod-form bacteria. Isolated bacteria showed various utilization of carbon sources such as glucose, maltose, lactose, xylose and arabinose, and showed tolerance at various range of salinity. It was isolated 82 genus in seawater and 114 genus in sediments. Dominant genera were *Pseudomonas, Vibrio, Flavobacterium* and *Acinetobacter* in seawater, and *Pseudomonas, Acinetobacter, Vibrio,* and *Mycobacterium* in sediments.

KEY WORDS □ heterotrophic marine bacteria, sediment, epifluorescence microscopy, dominant genera, biovolume

The intertidal zone near Kunsan of Yellow sea is a typical estuary. Along the length of the estuary, flood tides entering the estuary and ebb tides leaving cause the interface between the salt and fresh waters to move rhythmicallly twice a day. Due to its relatively shallow, protected waters, and the availability of nutrients, it showes exceptionally rich in biological activity and provides commercially most valuable marine products. The microbiological baseline studies in Kum River estuarine sediment near studying area were performed by Lee *et al.* (1986) and Lee (1987). However, few microbiological studies (Lee *et al.* 1989) were reported in this area. Recently river basin barrage was constructed and arranged estuary mouth area to promote ship harborring. Therefore, it is needed to understand the function and structure of this intertidal zone by the baseline survey to preserve this ecosystem. In this paper, we focus on the distribution and characteristics of heterotrophic marine bacteria in this ecosystem.

MATRERIALS AND METHODS

Sampling and enumeration of bacterial population

Samples were collected during the time from August, 1987 to July, 1988 (except January, 1988) in the vicinity of Kunsan, the western part of Korea. Samples were processed within a few hours of collection and samples were maintained at 5 °C during storing. Marine agar 2216 (Difco) was used for plating viable heterotrophic bacteria. For the determination of physiological bacteria groups, gelatin (0.4%) for proteolytic bacteria, tween 80 (0.1%) for lipolytic bacteria, and soluble starch (0.2%) for amylolytic bacteria were added, respectively, as a sole carbon source in the basal medium (Polczar, 1957). After incubation at 20 °C for 2 weeks, colonies were counted by the methods of Holding and Collee (1971). For direct counting, the staining procedure is essentially the same as given by Zimmermann (1977). The prepartion is then examined by epifluorescence microscopy (magnification × 1,500) (Bausch & Lomb). Bacterial cell volume was caculated as follows; Vol. of coccus= $4/3\,\pi r^3$, vol. of rod= πr^2 $(1 - 2/3\ r)$ (1: length of bacteria, r = radius).

Numerical taxonomic study

Isolates were tested for thirty five characters including morphorogical, physiological, and biochemical and nutritional features. Numerical taxonomic analyses were performed using single linkage clustering (Sokal and Sneath, 1963). Phenotypic groupings were generally recognized at greater than or equal to 70% similarity.

RESULTS AND DISCUSSION

The numbers of total saprophyte and physiologically characteristic bacteria in water represented in Fig. 1. Total saprophytic numbers in waters by plate counting showed distinct seasonal fluctuations, with maxima in early spring (especially in April) and late summer (July to September), while the minima occur during early summer (May to June) and late autumn (November to December). Physiologically characteristic bacteria curve also showed similar tendency. These indicate a strong correlation between saprophyte and phytoplankton development which, as a rule, begin in February for spring peak and in August for autumn peak (Lee *et al.*, 1989). Total numbers of saprophyte in sediments which were found on the ZoBell 2216 media were several orders of magnitude higher than in water samples (Fig. 2). The highest numbers were found at surface of sediment on August. They were between 1.12×10^4 and 4.78×10^6 c.f.u. per g dry sediment. The distribution of saprophytic number was strongly correlated with the sediment depth and overlying water temperature.

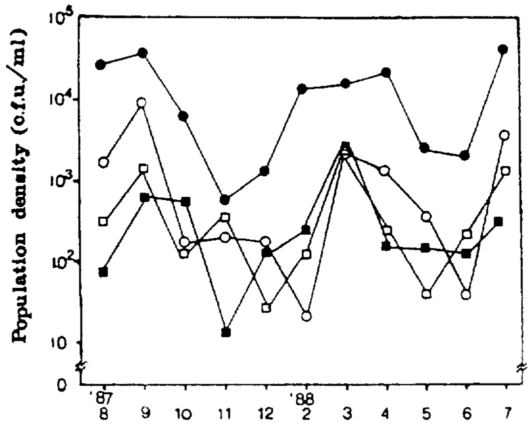

Fig. 1. *Monthly average distribution of total and physiologically characteristic bacteria in the intertidal waters near Kunsan of Yellow Sea, Korea* (●—● Total bacteria, ○—○ Proteolytic bacteria, ■—■ Lipolytic bacteria, □—□ Amylolytic bacteria).

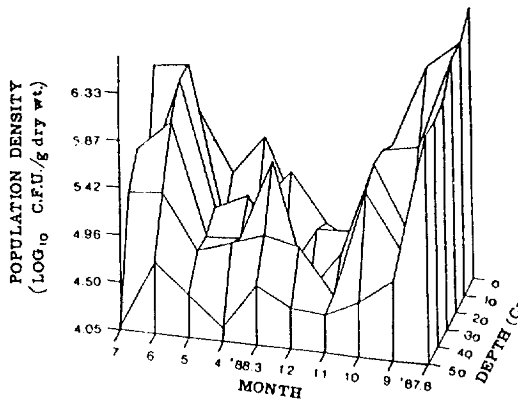

Fig. 2. *Three dimensional diagram of seasonal variation of heterotrophic marine bacteria at different depth of sediments*

Most of the cells counted using epifluorescence direct counting were free-living and about 80% were rod-shaped. High values appeared during summer (July) by both methods, but the lowest value appeared in November by plate count method, whereas by direct count method in March (Table 1). Great anomalies are apparent between direct and viable count, *i.e.* saprophyte numbers by direct count were about 240 -17,400 times higher than those of plate count to all months. Such difference of bacterial number was also obtained from several papers (Jannasch and Jones, 1959; Kogure *et al.*, 1979; Dahle and Laake, 1982; Simidu *et al.*, 1983; Staley and Konopka,

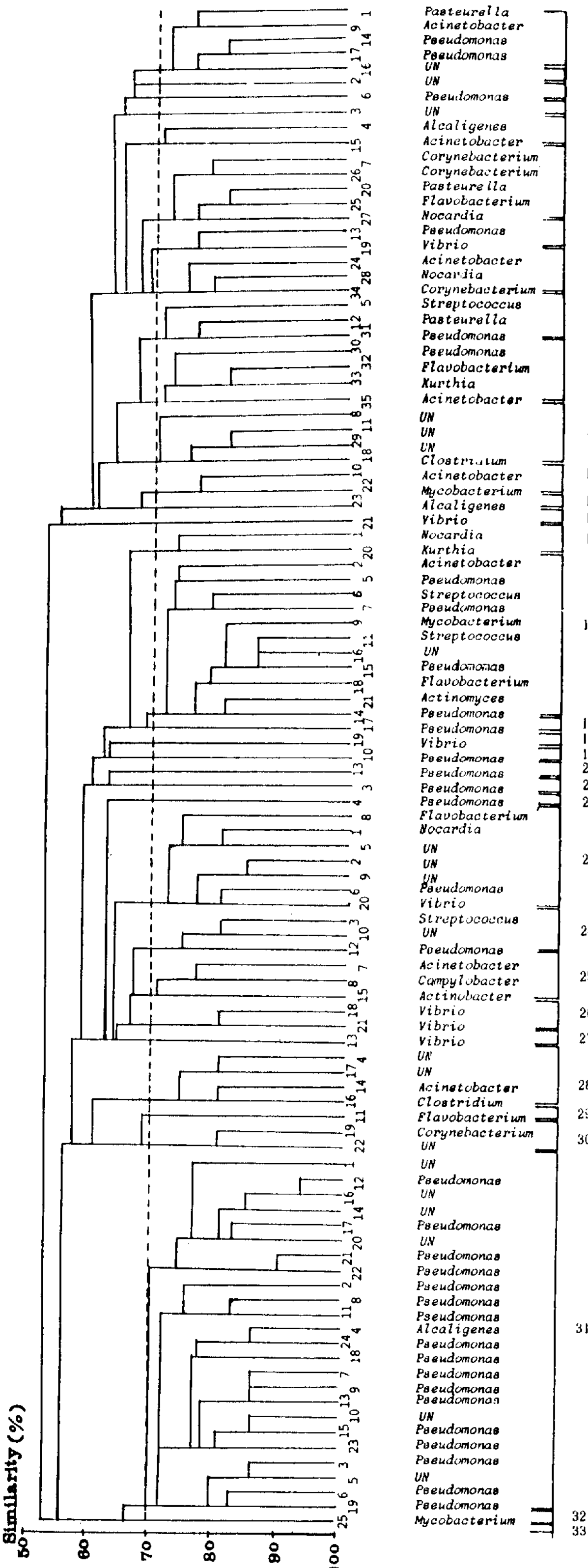

Fig. 3. Dendrogram of heterotrophic marine bacteria characters isolated in the intertidal waters near Kunsan of Yellow Sea, Korea.

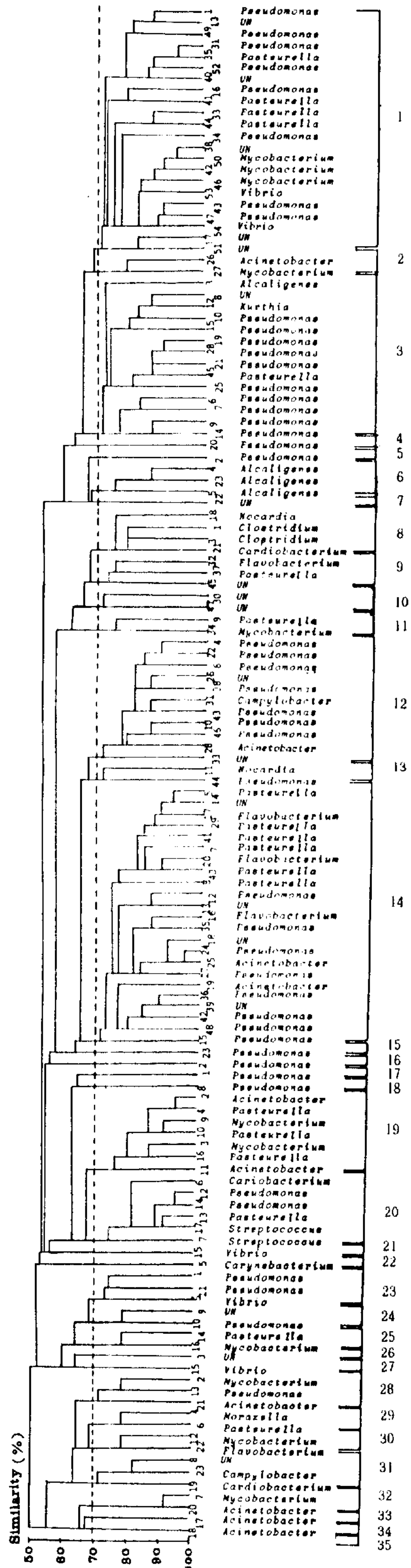

Fig. 4. Dendrogram of heterotrophic marine bacteria characters isolated from sediments near Kunsan of Yellow Sea, Korea

Table 1. *Seasonal fluctuation of total number, average biovolume and total biomass of heterotrophic marine bacteria in waters near Kunsan of Yellow Sea, Korea in 1987/88.*

Month	Cocci				Rods			
	Observed number	*Total number	Average biovolume ($\times 10^{-2} \mu m^3$)	**Total biomass	Observed number	*Total number	Average biovolume ($\times 10^{-2} \mu m^3$)	**Total biomass
Aug. 1987	1,316	3.8	4.69±0.74	17.8	3,543	10.4	5.25±0.19	54.6
Sep.	1,176	3.5	5.17±0.83	18.1	3,193	9.4	5.27±0.19	49.5
Oct.	1,258	3.7	3.60±0.58	13.3	2,730	8.1	4.69±0.18	38.0
Nov.	1,400	4.1	3.55±0.42	14.6	3,028	8.9	5.00±0.12	44.5
Dec.	983	2.9	5.33±0.68	15.5	2,277	6.7	6.20±0.18	41.5
Feb. 1988	876	2.6	4.86±0.68	12.6	2,971	8.8	7.79±0.36	68.6
Mar.	542	1.6	3.19±0.59	5.1	1,943	5.7	7.52±1.44	42.9
Apr.	1,213	3.6	3.82±0.44	13.8	3,161	9.3	12.94±0.21	120.3
May	597	1.8	4.80±0.71	8.6	3,056	9.0	4.76±0.18	42.8
Jun.	856	2.5	6.19±0.76	15.5	3,741	11.0	4.57±0.17	50.3
Jul.	1,087	3.2	4.69±1.55	15.0	13,603	40.1	10.14±0.22	406.6

*unit: $\times 10^6$ cells/m*l*, **unit: $\times 10^4 \mu m^3$/m*l*.

Table 2. *The morphological, physiological characteristics of heterotrophic marine bacteria in waters and sediments (% of population)*

Month / Characters	Water				Sediment			
	Aug.	Oct.	Mar.	May	Aug.	Oct.	Mar.	May
MORPHOLOGICAL								
Rod shaped	55	75	30	6	35	22	32	89
Coccus shaped	45	25	70	95	65	78	68	11
Gram negative	83	80	58	77	31	35	41	89
Motility	37	89	35	99	19	11	35	94
PHYSIOLOGICAL								
Growth at 25 °C	93	85	95	100	100	100	100	100
Growth at 37 °C	100	100	68	100	100	100	56	100
Growth at 42 °C	98	100	15	95	100	100	57	64
Growth on NA medium	100	100	100	100	100	100	100	100
Growth on SS medium	25	40	28	5	98	0	0	0
Growth on MacConkey medium	33	59	8	0	66	11	0	0
Growth on TCBS medium	20	5	46	5	16	54	44	50
Growth 0% NaCl	16	44	98	99	78	99	98	100
Growth 6% NaCl	100	100	100	100	18	98	59	98
BIOCHEMICAL								
Catalase activity	96	61	70	100	85	99	55	87
Oxidase activity	62	65	31	100	44	22	98	71
Starch hydrolysis	12	0	29	21	2	0	26	10
Gelatin liquefaction	85	93	74	100	34	59	100	69
MR	51	48	34	77	52	100	17	89

VP	8	40	70	28	99	100	30	25
Indole test	95	100	98	100	0	0	0	0
Acid from								
Glucose	95	100	100	76	81	0	100	100
Maltose	77	55	35	99	85	100	82	59
Lactose	49	57	2	78	70	55	2	23
Sucrose	95	75	75	99	84	100	81	87
Xylose	95	29	70	92	44	68	54	61
Arabinose	93	35	29	95	45	100	17	65
Gas production in glucose	0	1	0	0	0	1	0	1
Fermention of								
glucose and lactose	96	57	71	3	62	23	11	82
H_2S production	0	0	0	0	0	0	0	0

1985). The mean bacterial volume from samples in water was found to be $3.19 \pm 0.59 \times 10^{-2} - 6.19 \pm 0.76 \times 10^{-2}$ μm^3 for cocci, whereas $4.57 \pm 0.17 \times 10^{-2} - 12.94 \pm 0.21 \times 10^{-2}$ μm^3 for rods (Table 1). Such average biovolume values for esturine and coastal waters in Yellow Sea were almost corresponded with those of other investigators (Zimmermann, 1975, 1977; Ferguson and Rublee, 1976). It seemed that average biovolume showed also some seasonal fluctuation. However, we cannot explain why it showed seasonal fluctuation. It may reflect complex nutritional and physico-chemical variations within the surveying area. In addition to estimating the bacterial volumes, we have equated the data to biomass. Total biomass of investigating area amounted to $5.1 \times 10^4 \mu m^3/ml$ - $17.8 \times 10^4 \mu m^3/ml$ for cocci and $38 \times 10^4 \mu m^3/ml$ - $406.6 \times 10^4 \mu m^3/ml$ for rods (Table 1). Maximum values of total biomass appeared during spring (April) and summer (July), whereas minimum values occurred in fall (October) and winter (December). Beacterial total biomass fluctuated monthly to almost a small extent (except April and July). Some of the characteristics of the isolated becteria are summerized in Table 2. Fifty eight to 83.3% of isolates obtained from waters and 30.9 -89.4% from sediments were gram negative. A high percentage of isolates collected from water sample were rods during August and October, however, isolates from sediment were almost cocci during surveying periods except May. A greater percentage of isolates both waters and sediments were mesophiles and could grow at 20 -42°C. Most isolates also showed tolerance at broad range of salinity and showed various utilization of carbon sources such as glucose, maltose, lactose, xylse and arabinose. The analysis of 1,240 bacteria in waters and 1,328 bacteria in sediments which were isolated during surveying periods yielded in 33 groups in waters and 35 groups in sediments (Figs. 3,4). The diversity of these groups isolated both waters and sediments indicate high similarities between the populations of different season.

적 요

서해 군산 인근해역에서 조간대와 퇴적토를 대상으로 해양 종속영양세균의 년중 분포와 계절별 특성에 대하여 조사하였다. 해양 종속영양세균의 년간 분포는 해수에서는 $7.5 \times 10^2 - 1.1 \times 10^5$ c.f.u./ml 범주에서 변화하였으며 퇴적토에서는 $1.6 \times 10^4 - 4.8 \times 10^6$ c.f.u./g dry sediment 범주에서 변화하였다. 형광현미경에 의한 형태학적 분포를 측정한 결과 전 조사기간 중 간균이 74% 이상을 차지하였다. 또한 조사된 세균의 평균 생체량은 구균의 경우 $3.19 \pm 0.59 \times 10^{-2} - 6.19 \pm 0.76 \times 10^{-2}$ μm^3였으며, 간균은 $4.57 \pm 0.17 \times 10^{-2} - 12.94 \pm 0.21 \times 10^{-2}$ μm^3이었다. 동정된 균들은 glucose, maltose, lactose, xylose, arabinose를 탄소원으로 이용하였고 다양한 농도의 염분에 내성이 있었다. 해수에서는 82속이, 퇴적토에서는 114속이 동정되었고 우점속은 해수에서는 *Pseudomonas, vibrio, Flavobacterium*과 *Acinetobacter*이였고, 퇴적토에서는 *Pseudomonas, Acinetodacter, Vibrio, Mycobacterium*이었다.

ACKNOWLEDGEMENTS

These studies were supported by the Korean Science and Engineering Foundation (871-0407-013-1). The views expressed are not necessarily those of the Foundaton. The generosity of Dr. Sang-Jong Kim in providing the epifluorescence microscopy is gratefully acknowledged.

REFERENCES

1. **Dahle, A.B. and M. Laake,** 1982. Diversity dynamics of marine bacteria studied by immunofluorescent staining on membrane filters. *Appl. environ. Microbiol.,* **43**: 169-176.
2. **Ferguson, R.L., and P. Rublee,** 1976. Contribution of bacteria to standing crop of coastal plankton. *Limnol. Oceanogr.,* **21**: 141-145.
3. **Holding, A.J. and J.G. Collee,** 1971. Routine biochemical tests, *In*: Norris J. R. and D. W. Ribbons (eds.), Methods in Microbiology. Vol. 6A. pp. 1-32, Academic Press Inc. Ltd. London and New York.
4. **Jannasch, H.W. and G.E. Jones,** 1959. Bacterial populations in seawater as determined by different methods of enumeration. *Limnol. and Oceanogr.,* **4**: 128-130.
5. **Kogure, K., U. Simidu and N. Taga,** 1979. A tentative direct microscopic method for counting living marine bacteria. *Can. J. Microbiol.,* **25**: 415-420.
6. **Lee, G.H., Y.C. Hah and S.W. Hong.,** 1986. Distribution of heterotrophic bacteria and physicochemical characteristics of sediments in Kum River Estuary. *Kor. Jour. Microbiol.,* **24**: 308-316.
7. **Lee, G.H.,** 1987. Environmental factors affecting seasonal distribution of heterotropic bacteria in Kum River estuarine sediment. *Kor. Jour. Microbiol.,* **25**: 137-143.
8. **Lee, W.H., G.H. Lee, M.S. Choi and D.M. Lee.,** 1989. Phytoplankton and bacterioplankton in the intertidal and subtidal waters in the vicinity of Kunsan. *The J. Oceano. Soc. Korea.,* **24**: 157-164.
9. **Polczar, M.J., MR,** 1957. Manual of microbiological methods. McGraw-Hill Co. New York.
10. **Rheinheimer, G,** 1977. Microbial ecology of a brackish water environment. *In*: ecological studies.Vol 256. W. D. Billings *et al.* (eds.) Springer-Verlag Gerlin, Heidelberg, New York.
11. **Simidu, U., W.J. Lee and K. Kogure,** 1983. Comparison of different techniques for determining plate counts of marine bacteria. *Bull. Jap. Soc. Sci. Fish.,* **49**, 1199-1203.
12. **Sokal, R.R. and P.H.A. Sueath,** 1963. Principles of numerical taxonomy. San Francisco: Freeman.
13. **Staley, J.T. and A. Konopka,** 1985. Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Ann. Rev. Microbiol.,* **39**: 312-326.
14. **Zimmermann, R,** 1975. Entwicklung und Anwendung von rasterelekronenmikroskopischen Methoden zur Ermittlung der Bakterienmenge in Wasserporben. *Thesis Univ. Kiel.*
15. **Zimmermann, R,** 1977. Estimation of bacterial number and biomass by epifluorescence microscopy and scanning electron microscopy. *In*: G. Rheinheimer (ed.), Microbial ecology of a brackish water environment. Springer-Verlag Berlin, Heidelberg, New York.

(Received October 12, 1990)
(Accepted December 7, 1990)