Copyright ©

Kieler Meeresforsch., Sonderh. 8, 81-85. Kiel 1991

Seasonal distribution and character of heterotrophic marine bacteria in the intertidal zone of the Yellow Sea near Kunsan, Korea

G.-H. Lee and D.-M. Lee
Department of Biology, Kunsan National University
Miryong-dong, san 68, Kunsan City, Jeon-Buk Do, 573-360, Korea

Abstract

Annual distribution of heterotrophic marine bacteria and seasonal characteristics were investigated in the intertidal water and sediments of the Yellow Sea near Kunsan, Korea. The heterotrophic marine bacteria ranged from 7.5×10^2 to 1.1×10^5 CFU ml^{-1} in water and 1.62×10^4 to 4.78×10^6 CFU g^{-1} dry sediment. As for the morphological distribution measured by epifluorescence microscopy, rod-shaped bacteria were more than 74 % of all cells during the investigation period. Average biovolume of sampled bacteria ranged from $3.19 \pm 0.59 \times 10^{-2}$ to $6.19 \pm 0.76 \times 10^{-2}$ μm^3 for coccoid bacteria, and from $4.57 \pm 0.17 \times 10^{-2}$ to $12.94 \pm 0.21 \times 10^{-2}$ μm^3 for rod-shaped ones. Isolated bacteria showed utilization of various carbon sources such as glucose, maltose, lactose, xylose and arabinose, and tolerance to a range of salinities. In total 82 strains were isolated from seawater and 114 strains from sediments. Dominant genera were *Pseudomonas, Vibrio, Flavobacterium* and *Acinetobacter* in seawater, and *Pseudomonas, Acinetobacter, Vibrio* and *Mycobacterium* in sediments.

Introduction

The intertidal zone of the Yellow Sea near Kunsan is a typical estuary in the western part of Korea. Along the length of the estuary, flood tides entering and ebb tides leaving cause the interface between the salt and fresh waters to move rhythmically twice a day. Due to its relatively shallow, protected waters, and the availability of nutrients, it is exceptionally rich in biological activity and provides commercially most valuable marine products. However, recently a river basin barriage was constructed and arranged at the estuary mouth area to promote ship harbouring. Therefore, it is necessary to understand function and structure of the ecosystem of this estuary in order to preserve this ecosystem. In this paper, we focus on the distribution and characteristics of heterotrophic bacteria in the ecosystem.

Material and methods

Sampling and counting of bacteria

Samples were collected from August, 1987 to July, 1988 (except January, 1988). Samples were processed within a few hours after collection and were maintained at 5 °C during storage. Marine agar 2216 (Difco) was used for plating viable heterotrophic bacteria. For the determination of physiological groups of bacteria 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 the sole carbon source to 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 that given by ZIMMERMANN (1977). The preparation is then examined by epifluorescence microscopy (magnification x 1500). Bacterial cell volume was calculated as follows: vol. coccus = $4/3\ \pi\ r^3$, vol. of rod = $\pi\ r^2\ (l - 2/3\ r)$ (l = length of bacteria, r = radius).

Numerical taxonomic study

Isolates were used for thirty five tests including morphological, physiological, biochemical and nutritional characteristics. Numerical taxonomic analyses were performed using single linkage clustering (SOKAL and SNEATH 1963). Phenotypic grouping was generally recognized at greater than or equal to 70 % similarity.

Results and discussion

Total saprophytic numbers showed distinct seasonal fluctuations, with maxima in early spring (especially in April) and late summer (July to September), while the minima occurred during early summer (May to June) and late autumn (November to December). The specialized bacteria also showed a similar tendency. This indicates a strong correlation between saprophytes and phytoplankton development, which as a rule begins in February for the spring peak and in August for the autumn peak (LEE et al. 1989). Total saprophyte numbers on marine agar (ZoBell) 2216 in sediments were several orders of magnitude higher than in water samples. The highest numbers were found at the surface of sediment in August. They were between 1.12×10^4 and 4.78×10^6 CFU per g dry sediment. The distribution of saprophyte numbers was strongly correlated with the sediment depth and overlying water temperature. Most of the cells counted using epifluorescence microscopy were free-living and about 80 % were rod-shaped. High values appeared during summer (July) by both methods, but lowest values appeared in November by plate count method, whereas the direct count method yielded lowest numbers in March (Table 1). Great differences are apparent between direct and viable counts, i.e. direct counts were about 240-17400 times higher than saprophyte numbers by plate counts. Such difference of bacterial numbers was also described by other authors (JANNASCH and JONES 1959, KOGURE et al. 1979, DAHLE and LAAKE 1982, RHEINHEIMER 1977, SIMIDU et al. 1983, STALEY and KONOPKA 1985). The mean bacterial volume from water samples 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 estuarine and coastal waters in the Yellow Sea were similar to those of other investigators (ZIMMERMANN 1975, 1977, FERGUSON and RUBLEE 1976). It seemed that average biovolume showed also some

Table 1. Seasonal fluctuation of saprophyte colony forming units (CFU), total numbers, average biovolumes and total biomass of bacteria in water of the Yellow Sea near Kunsan in 1987/88.

month	Saprophyte number (CFU ml^{-1})	total number ($x10^6$cells ml^{-1})	average biovolume ($x10^{-2}\mu m^3$)	total biomass ($x10^4$ μm^3 ml^{-1})
		Cocci		
Aug. 1987	1316	3.8	4.69 ± 0.74	17.8
Sep.	1176	3.5	5.17 ± 0.83	18.1
Oct.	1258	3.7	3.60 ± 0.58	13.3
Nov.	1400	4.1	3.55 ± 0.42	14.6
Dec.	983	2.9	5.33 ± 0.68	15.5
Feb. 1988	876	2.6	4.86 ± 0.68	12.6
Mar.	542	1.6	3.19 ± 0.59	5.1
Apr.	1213	3.6	3.82 ± 0.44	13.8
May	597	1.8	4.80 ± 0.71	8.6
Jun.	856	2.5	6.19 ± 0.76	15.5
Jul.	1087	3.2	4.69 ± 1.55	15.0
		Rods		
Aug. 1987	3543	10.4	5.25 ± 0.19	54.6
Sep.	3193	9.4	5.27 ± 0.19	49.5
Oct.	2730	8.1	4.69 ± 0.18	38.0
Nov.	3028	8.9	5.00 ± 0.12	44.5
Dec.	2277	6.7	6.20 ± 0.18	41.5
Feb. 1988	2971	8.8	7.79 ± 0.36	68.6
Mar.	1943	5.7	7.52 ± 1.44	42.9
Apr.	3161	9.3	12.94 ± 0.21	120.3
May	3056	9.0	4.76 ± 0.18	42.8
Jun.	3741	11.0	4.57 ± 0.17	50.3
Jul.	13603	40.1	10.14 ± 0.22	406.6

seasonal fluctuations. However, we cannot explain why. It may reflect the complex nutritional and physico-chemical variations within the surveying area. From the bacterial volumes we have estimated the total biomass expressed as total bacterial cell volume. Total biomass of the investigated area amounted to 5.1 x 10^4 μm^3 ml^{-1} - 17.8 x 10^4 μm^3 ml^{-1} for cocci and 38 x 10^4 μm^3 ml^{-1} - 406,6 x 10^4 μm^3 ml^{-1} for rods (Table 1). High values of total biomass appeared during spring (April) and summer (July), whereas minimum values occurred in fall (October) and winter (December). Bacterial total biomass fluctuated monthly to a small extent (except April and July). Some of the characteristics of the isolated bacteria are summarized in Table 2. 58 - 83.3 % of isolates obtained from water and 30.9 - 89.4 % from sediment were Gram negative. A high percentage of isolates collected from water samples were rods during August and October, however, isolates from sediment were mostly cocci during the investigation period except in May. A large percentage of isolates from both water and sedi-

Table 2. The morphological, physiological and biochemical characteristics of heterotrophic bacterial population in water and sediment (% of the population).

Characters \ Month	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	97
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
Fermentation of glucose and lactose	96	57	71	3	62	23	11	82
H_2S production	0	0	0	0	0	0	0	0

ment were mesophiles and could grow at 20 - 42 °C. Most isolates also showed tolerance to a broad range of salinity and were able to utilize various carbon sources such as glucose, maltose, lactose, xylose and arabinose. The analysis of 1240 bacteria in water and 1328 bacteria in sediment, which were isolated during the investigation period yielded 33 groups in water and 35 groups in sedi-

ment. The diversity of these groups isolated from both water and sediment indicate high similarities between the populations of different seasons.

References

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.

FERGUSON, R.L. and P. RUBLEE, 1976. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. 21, 141-145.

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. Academic Press Inc. Ltd. London and New York. 1-32.

JANNASCH, H.W. and G.E. JONES, 1959. Bacterial populations in seawater as determined by different methods of enumeration. Limnol. Oceanogr. 4, 128-130.

KOGURE, K., U. SIMIDU and N. TAGA, 1979. A tentative direct microscopic method for counting living marine bacteria. Can. J. Microbiol. 25, 415-420.

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. J. Ocean. Soc. Korea 24, 157-164.

POLCZAR, M.J., 1957. Manual of microbiological methods. McGraw-Hill Co. New York.

RHEINHEIMER, G., 1977. Microbial ecology of a brackish water environment. Springer-Verlag Berlin, Heidelberg, New York.

SIMIDU, U., W.J. LEE and K. KOGURE, 1983. Comparison of different techniques for determining plate counts of marine bacteria. Bull. Japan. Soc. Sci. Fisheries 49, 1199-1203.

SOKAL, R.R. and P.H.A. SNEATH, 1963. Principles of numerical taxonomy. Freeman, San Francisco.

STALEY, J.T. and A. KONOPKA, 1985. Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Ann. Rev. Microbiol. 39, 321-326.

ZIMMERMANN, R., 1975. Entwicklung und Anwendung von rasterelektronenmikroskopischen Methoden zur Ermittlung der Bakterienmenge in Wasserproben. Doctoral Thesis Univ. Kiel.

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.