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Distribution, cell volume and extracellular enzyme activities of heterotrophic bacteria near the mouth of Keum River, Korea.

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INTRODUCTION

A river-mouth is a characteristic ecosystem by variety of gradients in physicochemical parameter, which may influence the activity of heterotrophic bacteria. Keum River, which flows into the Yellow Sea, is located in the middle of Korean Peninsula. Keum River plays an extremely important role not only for domestic, industrial, and agricultural purposes, but also for recreational waters and a biotype of flora and fauna. However, owing to a recent construction of river basin barriage and the excess discharge of domestic sewage and industrial effluents, the water quality in river-mouth became worse than ever. The numbers of heterotrophic bacteria and saprophytic bacteria serve as trophic lever indicators[11]. But information on microbiological water quality in this area is not sufficient[3, 5, 7, 8]. Therefore, we carried out this study to understand the spatial variation and function of heterotrophic bacterial populations near Keum River-mouth environment.

METHODS

Water samples for the measurement of bacterial cell volume and for the colony forming units(cfu) of heterotrophic bacteria were collected aseptically 4 times from November, 1992 to Auguest, 1993 at 5 stations(Fig. 1). Station(St.) 1, with an average depth of 5 m, is located near the river basin barriage, where seawater no more flows into river, St. 2 is located near the harbour, where domestic sewage flows out from tributary and St. 3, 4, 5 are located near the mouth of river, where the depth is about 10 m. Marine agar 2216(Difco) was employed as medium for plating viable heterotrophic bacteria. For the determination of physiological groups of heterotrophic bacteria, gelatin(0.4 %) for proteolytic bacteria, tween 80(0.1 %) for lipolytic bacteria, and soluble starch(0.2 %) for amylolytic bacteria were added, respectivety, as the sole carbon source to the basal medium[9]. After incubation at 20℃ for 2 weeks, colonies were counted by the methods of Holding and Collee(1971,[1]). Bacterial cell volumes were measured with a epifluorescence microscope using acridine orange as dye[12]. Bacterial cell volumes were caculated as fllows: Vol. of coccus $= 4/3\pi r^3$, vol. of rod $= \pi r^2(1 - 2/3 r)$(l: length of bacteria, r = radius). The extracellular enzyme activities of N-acethyl-β-D-glucosaminidase (chitinase), α-glucosidase(amylase), β-glucosidase, cellulase, phosphatase were determined by the method of Kim and Hoppe(1986,[2]).

RESULTS AND DISSCUSSION

Heterotrophic bacterial numbers fluctuated between 3.3×10^3 to 3.41×10^5 cfu/ml during surveying periods and exhibited a pronounced seasonal and spatial variation(Fig. 2). Heterotrophic bacterial numbers varied during November, 1992 to May, 1993 at all stations, but the highest number($1.81 \times 10^5 \sim 3.41 \times 10^5$ cfu/ml) was observed in August, 1993 for every station. Station 1 and 2, where domestic sewage flowed out from tributary, showed the hightest heterotrophic bacterial numbers among all stations.

The seasonal fluctuation in numbers of specialized physiological groups of bacteria at sampling stations also showed a similar tendency as heterotrophic bacteria and their tendency agreed well with the data investigated by Lee and Lee(1991,[4]) in the intertidal zone of the Yellow Sea near Kunsan. The population densities were ranged from 8.5×10^2 to 3.53×10^4 cfu/ml for proteolytic bacteria, from 8.5×10 to 4.69×10^3 cfu/ml for amylolytic bacteria, and from 10 to 2.37×10^3 cfu/ml for lipolytic bacteria(Fig. 3a, 3b, 3c). The numbers of specialized physiological groups of bacteria seemed to respond fast to a change in environmental conditions. The proportion of specific functional group of bacteria was higher in the order of proteolytic, amylolytic, and lipolytic bacteria. The percentage of proteolytic bacteria related to total heterotrophic numbers ranged between 10.4 and 25.8 %, whereas the percentage of amylolytic and lipolytic bacteria to the total heterotrophic bacteria ranged between 1.4 ~ 2.6 % and 0.3 ~ 0.7 %, respectively. Such a high proportion of proteolytic bacteria seems to be interrelated with characteristics of pollution and may reflect complexity in nutritional variation in this surveying area.

The mean bacterial cell volume was found to be between $7.45 \pm 0.11 \times 10^{-2}\ \mu m^3$ and $8.18 \pm 0.32 \times 10^{-2}\ \mu m^3$ for cocci, and between $6.87 \pm 0.35 \times 10^{-2}\mu\ m^3$ and $7.61 \pm 0.27 \times 10^{-2}\mu\ m^3$ for rods(Table 1). Relatively higher values were measured in November, 1992 compared with other sampling periods. Average cell volume in sampling stations was similar with the cell volume in the intertidal zone of the Yellow Sea near Kunsan observed by Lee and Lee(1991,[4]). The cell volume measured in this sampling stations were usually higher than those reported by Watson et al.(1977,[11]). The reason might be the combined effect of environmental condition and a greater availability of easily degradable organic compounds[10] near sampling stations. It seemed that average cell volume showed also some seasonal fluctuation, but no distinct pattern was shown in annual variation. And no distinct correlationship was detected between bacterial numbers and average cell volumes.

Seasonal variation of extracellular enzyme activities near Keum River-mouth varied as in the upper part of the river(St.1 ~ St.2) as in the lower part of river(St.3 ~ St.5), and their measured values were higher than those of intertidal zone near Kunsan[6]. Extracellular enzyme activities ranged from 0 to 3.5 μ M/l/hr for glucosidase(Fig. 4a), from 0.02 to 1.9 μ M/l/hr for amylase(Fig. 4b), from 0.01 to 1.4 μ M/l/hr for cellulase(Fig. 4c), from 0.01 to 1.25 μ M/l/hr

for chitinase(Fig. 4d), from 0 to 9.9 μ M/l/hr for phosphatase(Fig. 4e). Extracellular enzyme activities near Keum River-mouth also showed seasonal variation and a marked fluctuation between stations. Higher values were recorded in summer(August, 1993) than other sampling periods. Among measured extracellular enzymes, phosphatase showed the highest activity, whereas chitinase showed the lowest. Extracellular enzyme activities peak close to St.1 and St.2, probably reflecting a greater abundance of organic nutrients in these stations. Seasonal bacterial extracellular enzyme activities were coincided with the changes in water temperature and heterotrophic bacterial numbers, but no significant correlation was detected between bacterial cell volumes and extracellular enzymes activities. Any conclusion drawn from these results must obviously be limited, so further studies are necessary to understand the function of heterotrophic bacterial populations near the mouth of Keum River environment.

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Table 1. Cell shape and observed number of samples for the determination of seasonal variation of bacterial cell volumes near Keum River-mouth in the middle of the Korean peninsula

Month	Coccus		Rod	
	Observed number (%)	Average cell volume ($\times 10^{-2}\mu$ m^3)	Observed number (%)	Average cell volume ($\times 10^{-2}\mu$ m^3)
Nov.	771 (21)	8.18 ± 0.32	2,838 (79)	7.61 ± 0.27
Mar.	652 (42)	7.62 ± 0.05	902 (58)	7.32 ± 0.41
May	1,842 (58)	7.45 ± 0.11	1,324 (42)	6.87 ± 0.35
Aug.	1,921 (35)	8.10 ± 0.67	2,938 (61)	7.28 ± 0.42

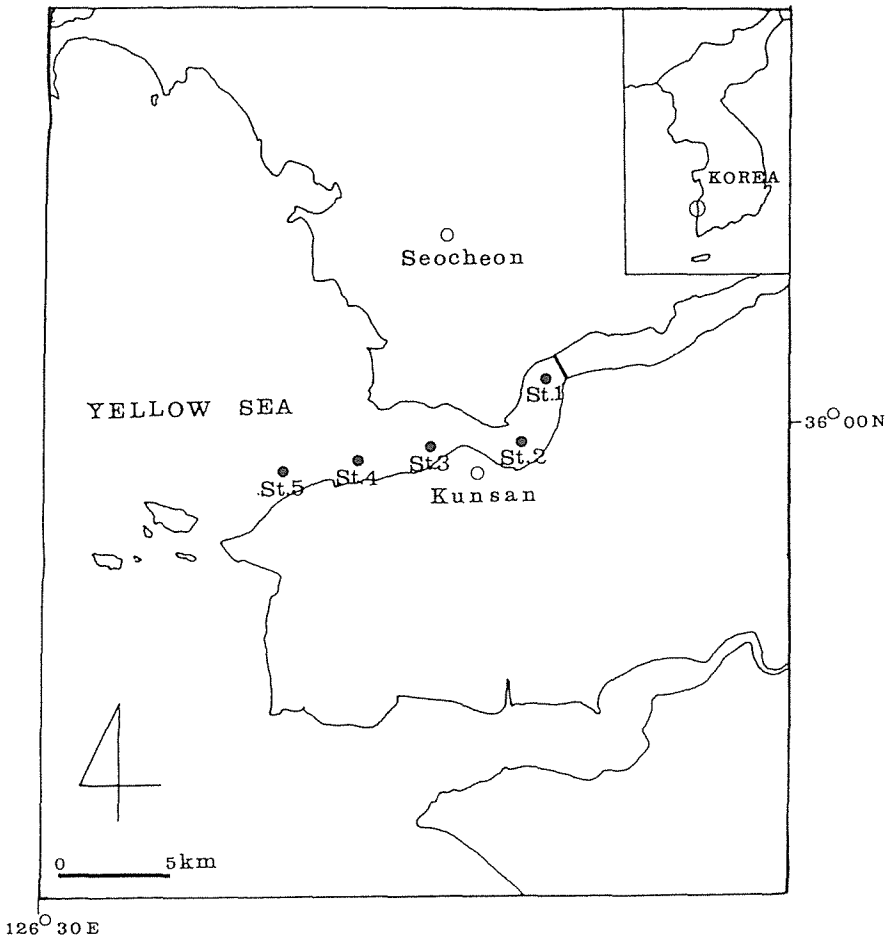

Fig. 1. A map near the mouth of Keum River showing the sampling stations.

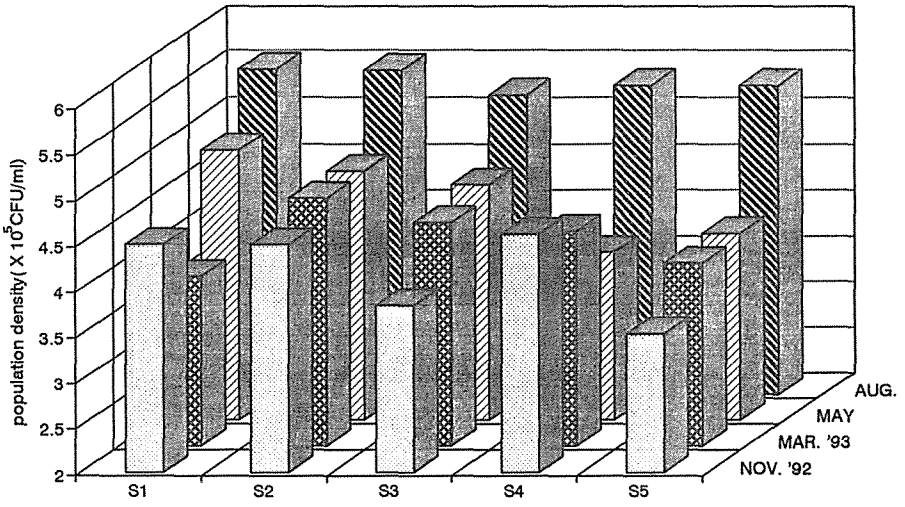

Fig. 2. The variation of total heterotrophic bacterial number from November, 1992 to August, 1993 near the mouth of Keum River in the middle of the Korean peninsula.

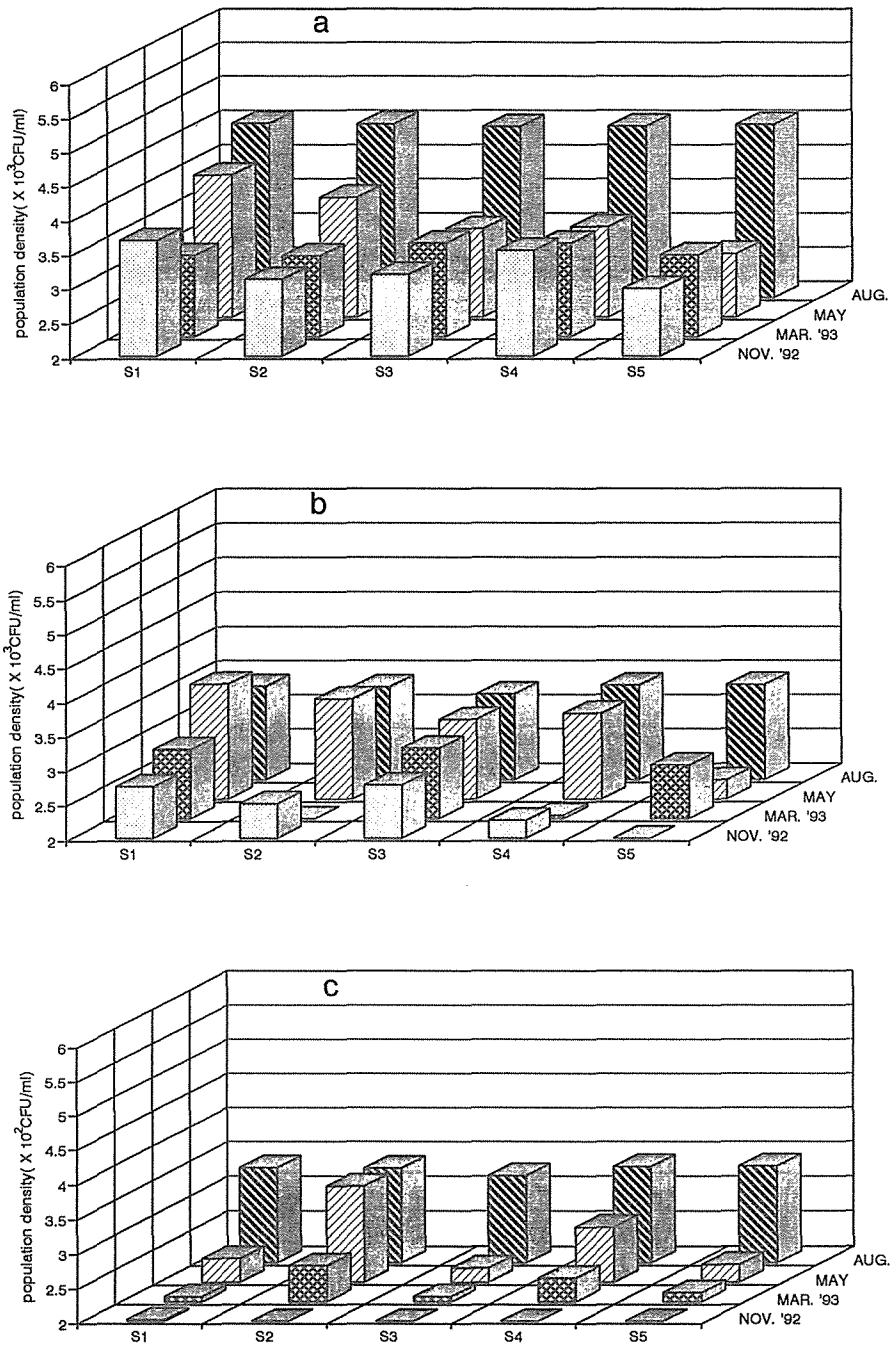

Fig. 3. Variations of proteolytic(a), amylolytic(b), and lipolytic bacteria(c) from November, 1992 to August, 1993 near the mouth of Keum River in the middle of the Korean peninsula.

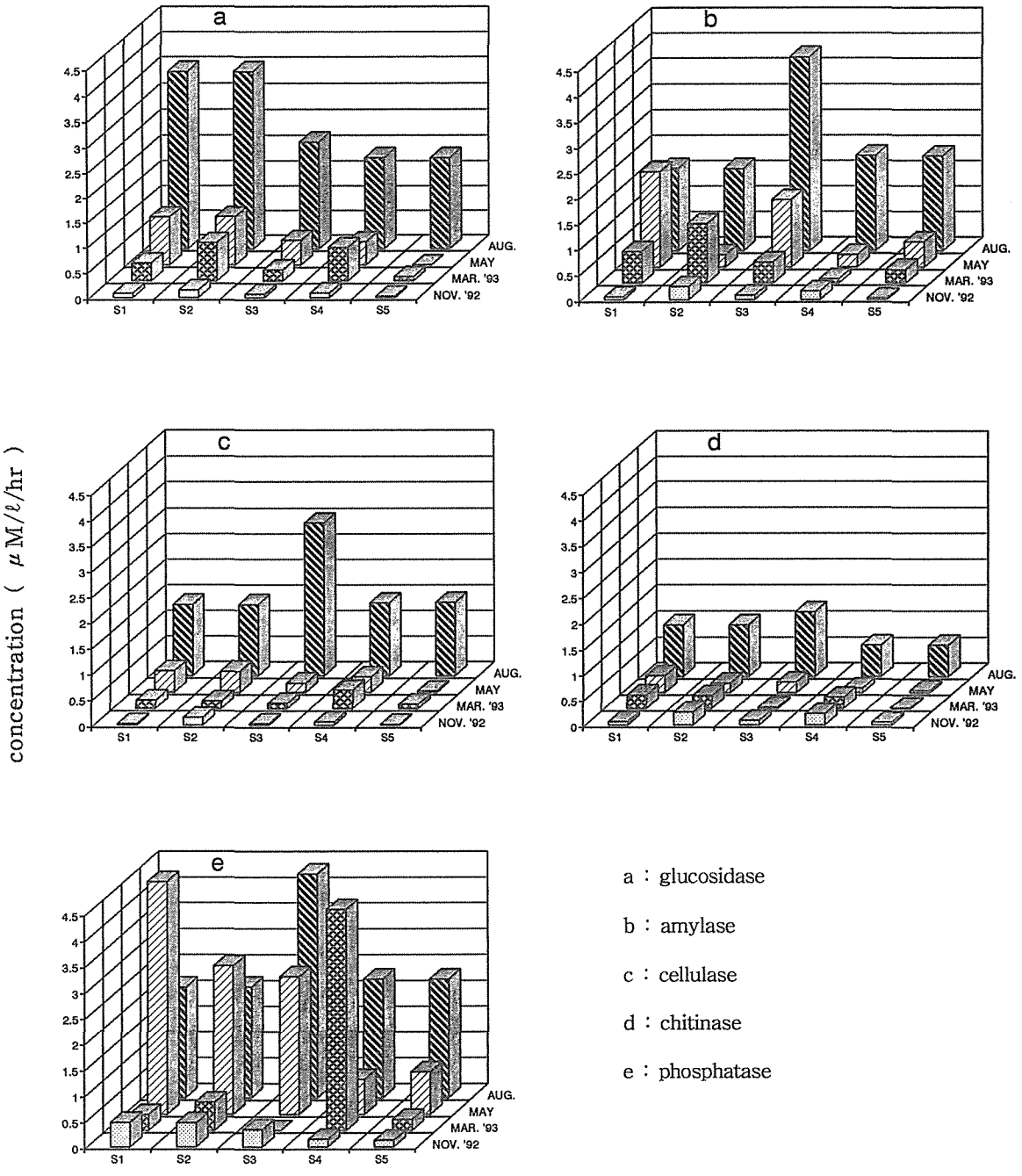

Fig. 4. The variation of extracellular enzyme activities(a:glucosidase, b:amylase, c:cellulase, d:chitinase, e:phosphatase).