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Numerical Taxonomy of Heterotrophic Bacteria in Naktong Estuary

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낙동강 하구에 분포한 종속영양세균의 수리학적 분류

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ABSTRACT: Using the numerical clustering method, 14 clusters (containing three or more strains) among 231 bacterial isolates from St. 1, 17 clusters among 252 isolates from St. 2, 17 clusters among 301 isolates from St. 3, and 15 clusters among 260 isolates from St. 4 were found at the 70% similarity value. The predominant organisms were identified as genera *Aeromonas, Vibrio,* and *Alcaligenes* at St. 1, *Alcaligenes* at St. 2, *Aeromonas, Vibrio,* and *Moraxella-Acinetobacter* group at St. 3, and *Pseudomonas* at St. 4.

KEY WORDS□numerical taxonomy, heterotrophic bacteria, Naktong Estuary

Heterotrophic bacteria reacts more quickly to the changes in their environment than the others and reflects the contents of easily degradable organic matter in the water(Rheinheimer, 1977). Thus heterotrophic bacteria can act as a good indicator of organic pollution load of the water at a given time, and they have been used recently to evaluate the effects arising from massive oil and other catastrophic events (Colwell and Walker, 1977; Tagger *et al.*, 1983).

The use of numerical methods for classification purposes is based on the assumption that an organism, a population, and a community can be expressed in numerical terms describing the characteristic features, and all characters have equal weight. Sneath and Sokal (1973) have pointed out that the use of numerical methods for taxonomic purposes has generally been more readily acceptable and has provoked far less criticism when applied to bacteria than to other organisms.

Naktong River goes through the southeast area of the Korean Peninsula making up a Kimhae delta, and finally flows into the southern sea of Korea. Cities, farmlands, and industrial complexes are dotted along Nakt ng River making it the major water resources not only for irrigation but also for industry. Since her basin area has very unfavorable conditions as for the water resources, river basin barrage was constructed to prevent water loss and to meet the demand for resources by blocking the influx of seawater at 1987. Therefore, the ecosystem of Naktong Estuary will be soon changed in a large scale. In this study, we investigated the characterization of the predominant bacterial communities before the construction of river basin barrage. It could be used as the baseline data of microfloral changes after the construction of barrage in Naktong Estuary.

MATERIALS AND METHODS

Sample collection

Four stations located in Naktong Estuary were sampled between July 1985 and October 1986

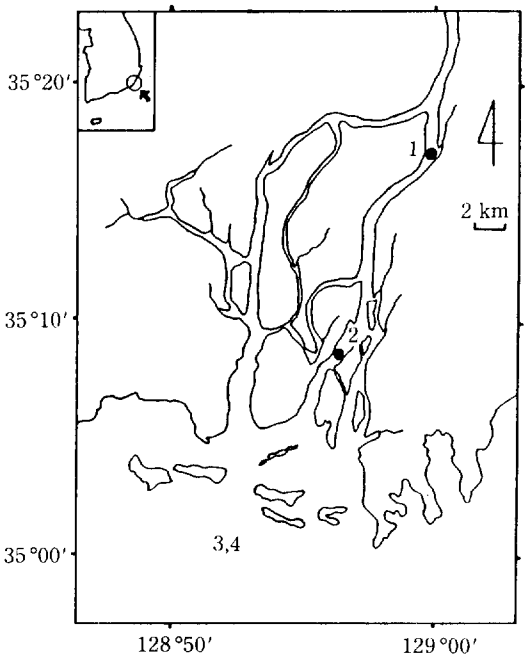

Fig. 1. *Map showing the sampling stations in Naktong Estuary.*

once a month (Fig. 1). Samples were collected about 1 meter under the water surface at St. 1, 2, 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. Each sample was placed in a rinsed 2,000 m*l* polyethylene bottle for the analysis of water quality and in a sterilized 100 m*l* glass bottle for bacterial examinations. Samples were kept on ice for transport to the laboratory and assayed within 8 hours of collection.

Isolation and characterization of bacterial strains

After calculating the densities of heterotrophic bacteria on three kinds of medium (N-0, N-10, Z-25), agar plate having the maximal number at each station was selected. More detailed descriptions of medium composition and procedure were described in the previous report (Kwon *et al.*, 1987). In order to simulate the effect of different concentrations of organisms in equal volume samples from different stations, the number of colonies selected was not the same for all stations, but was in direct proportion to the number of colonies appearing on the plates. Thus, the number of isolates was proportional to the size of bacterial populations at each station. All isolates were pure-cultured and maintained on agar slants or plates. A total of 72 morphological and physiological features were examined for each isolate (Kwon *et al.*, 1987).

Clustering of isolates and classification of clusters

Cluster analysis was carried out according to the clustering method of single linkage clustering (Sneath and Sokal, 1973); two strains or two groups are linked together at the highest similarity value which is shared by two members of these groups. Clusters containing three or more strains at 70% similarity value were subject to examine, but those occured as single-or two-membered were discarded.

Classification of clusters to generic level was performed by refering the text descriptions in "Bergey's Manual of Systematic Bacteriology, Vol. 1 and 2" (Krig and Holt, 1984; Sneath *et al.*, 1986) and "The Prokaryotes" (Starr *et al.*, 1981), and the taxonomic keys (Shewann *et al.*, 1960; Bonde, 1977; LeChevallier *et al.*, 1980).

RESULTS

Environmental parameters in Naktong Estuary are summarized and shown in Table 1. The concentrations of nutrients were high at upper stream and slow down with going to downstream of the investigated area.

Since freshwater and seawater are mixed in estuary, the density and diversity of heterotrophic bacteria appeared on agar plates are varied with regard to the salt concentrations of media. Thus, the selection of appropriate media is determined at first to isolate bacterial strains. At St. 1, average bacterial density on N-0 medium was the maximum. At St. 2, the maximum was found on N-10 medium, but at St. 3 and 4 on Z-25 medium (Table 2). Isolation media which have more colonies than the others may be said that they are less selective and more diverse bacterial flora can grow on them. Therefore, bacterial strains were isolated from N-0 medium at St. 1, from N-10 me-

Table 1. *Summary of environmental parameters at each station of Naktong Estuary.*

	St. 1				St. 2				St. 3				St. 4			
	max	min	mean	SD§	max	min	mean	SD	max	min	mean	SD	max	min	mean	SD
temp*(°C)	30.0	4.2	15.4	8.5	30.0	5.5	15.7	7.3	27.5	6.0	16.6	5.6	27.5	7.0	16.0	5.1
pH	7.7	6.2	6.9	0.5	7.9	6.5	7.2	0.5	8.2	6.8	7.9	0.3	8.2	7.4	7.9	0.2
sal**(‰)	7.2	0.0	1.7	2.5	20.1	0.0	9.3	7.4	28.8	12.0	22.1	5.9	32.2	22.0	27.4	2.7
DO (mg/*l*)	8.8	3.4	6.5	1.7	9.3	4.0	6.5	1.6	9.8	4.2	7.1	1.7	10.3	3.5	7.1	1.9
BOD (mg/*l*)	9.9	0.8	4.5	2.4	9.2	1.5	4.5	2.3	9.0	0.8	4.0	2.6	9.0	1.0	3.6	2.4
MBOD (mg/*l*)	200	18	69	55	200	4	68	62	150	10	58	44	110	8	45	33
MBOD-N(mg/*l*)	1540	32	594	365	1000	31	561	309	920	26	495	297	920	32	499	279
MBOD-P(mg/*l*)	235	10	81	70	186	8	70	50	192	14	67	47	184	10	55	46
NH_4^+(μg-N/*l*)	1082	31	204	263	430	53	194	120	380	4	78	91	616	4	84	150
NO_2^-(μg-N/*l*)	79	3	42	24	86	12	42	20	52	0	17	16	80	0	15	19
NO_3^-(μg-N/*l*)	2294	327	861	555	2600	125	583	610	845	13	158	215	544	12	135	148
PO_4^{-3}(μg-P/*l*)	157	12	26	19	62	8	36	15	76	1	36	19	63	8	30	14
chl# (mg/m^3)	30.7	0.4	13.4	10.3	22.6	1.7	11.2	6.5	15.4	1.7	5.3	3.6	10.3	1.1	5.0	3.3

§standard deviation, *water temperature, **salinity, #chlorophyll a

Table 2. *Variations of heterotrophic bacterial densities (× 10^4 CFU/ml) on 3 kinds of medium differing salt concentrations in Naktong Estuary.*

	N-O		N-10		Z-25	
	range	mean	range	mean	range	mean
St. 1	1.37 -51.0 (86.10.27)* (86. 3.27)	13	0.83 -35.0 (86.10.31) (86. 1.23)	9.6	0.063-6.87 (85.10.27) (85.12.27)	2.3
St. 2	1.23 -392 (86. 5.25) (85. 7.26)	36	3.3 -507 (85.12. 1) (85. 7.26)	40	1.07 -26.0 (85.10.27) (86. 3.27)	6.2
St. 3	0.094-3.3 (86. 5.25) (85. 8.15)	1.3	0.085-10.1 (86. 5.25) (86. 9.25)	1.8	0.31 -13.1 (85.10.27) (86. 6.27)	2.1
St. 4	0.033-1.33 (86. 8. 5) (85. 8.15)	0.44	0.08 -1.52 (86. 8. 5) (86. 6.27)	0.47	0.108-4.23 (86. 1.23) (85. 8.15)	1.1

* (year. month. day)

dium at St. 2, and from Z-25 medium at St. 3 and 4 by random selection and examined the characters.

231 strains were isolated from St. 1, 252 strains from St. 2, 301 strains from St. 3, and 260 strains from St. 4, and grouped by cluster analysis based on similarity value of 70% at each station. By these procedures, 14 clusters could be found among 225 isolates from St. 1, 17 clusters among 243 isolates from St. 2, 17 clusters among 265 isolates from St. 3, and 5 clusters among 255 isolates from St. 4 (Fig. 2, 3, 4, 5).

Among all clusters, the distinct features of major clusters with more than 10% of total isolates at each station are shown in Table 3. Based on the features, the major clusters of each station can be summarized as following 7 generic groups:

1. Gram-negative, fermentative, oxidase-positive, motile rods (clusters of 10, 11, 12 of St. 1, cluster 14 of St. 2, clusters 3, 12, 13 of St. 3, cluster 6 of St. 4). These clusters were similar to genera *Aeromonas* and *Vibrio* (Bonde, 1977; LeChevallier *et al.*, 1980; Shewann *et al.*, 1960).

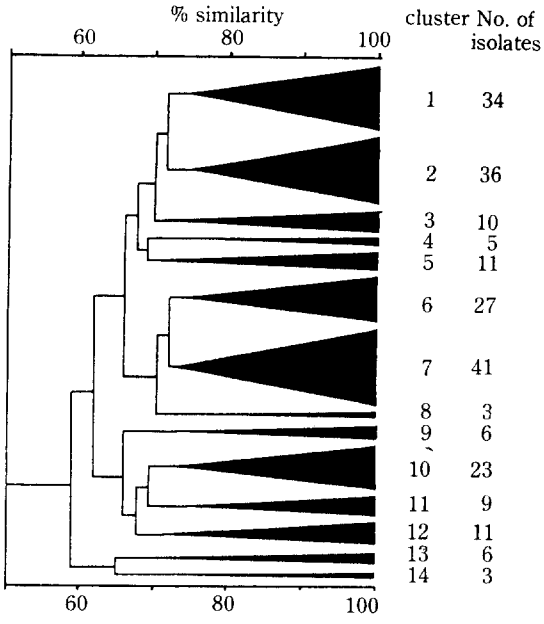

Fig. 2. *Simplified dendrogram of percent similarity showing relationships among all isolates from St. 1.*

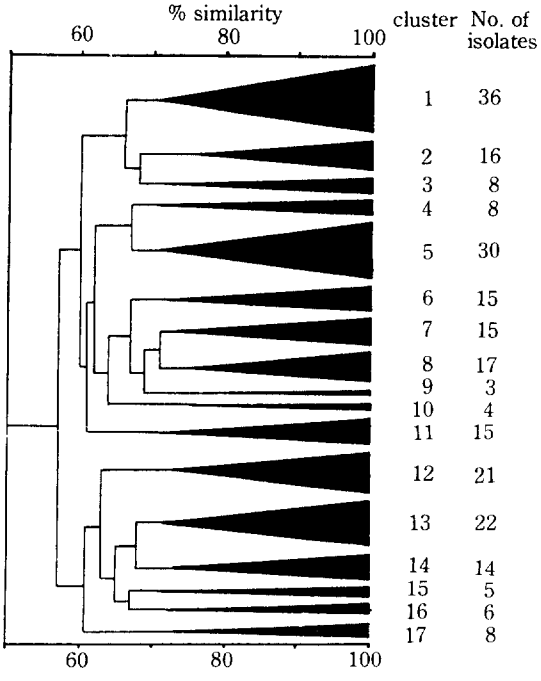

Fig. 3. *Simplified dendrogram of percent similarity showing relationships among all isolates from St. 2.*

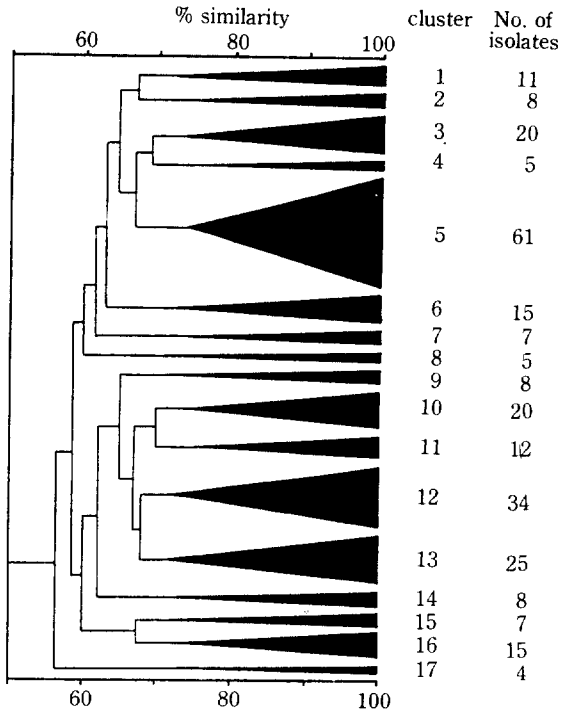

Fig. 4. *Simplified dendrogram of percent similarity showing relationships among all isolates from St. 3.*

Aeromonas and *Vibrio* could be differentiated by vibriostatic agent, 0/129, the strains of the former being resistent while those of the latter being sensitive. This generic group was predominant especially at St. 1 and 3.

2. Gram-negative, oxidative or non-fermentative, motile rods (cluster 1 of St. 1, cluster 7 of St. 2, clusters 1, 6 of St. 3, clusters 3, 4, 11, 15 of St. 4). The strains in these clusters belonged to the genus *Pseudomonas* (Shewann *et al.*, 1960; LeChevallier *et al.*, 1980). Among those clusters of St. 4, strains of cluster 3 were not able to grow in 0% of NaCl and 1.6% of KCl, that is, showed the characteristics of marine bacteria. But strains of clusters 4, 11, and 15 could grow not only in the above mentioned salt concentrations but also in 5% of NaCl, which could be euryhaline strains. This generic group was especially dominant at St. 4.

3. Gram-negative, non-motile, non-fermentative, oxidase-positive (cluster 7 of St. 1, clusters 1,

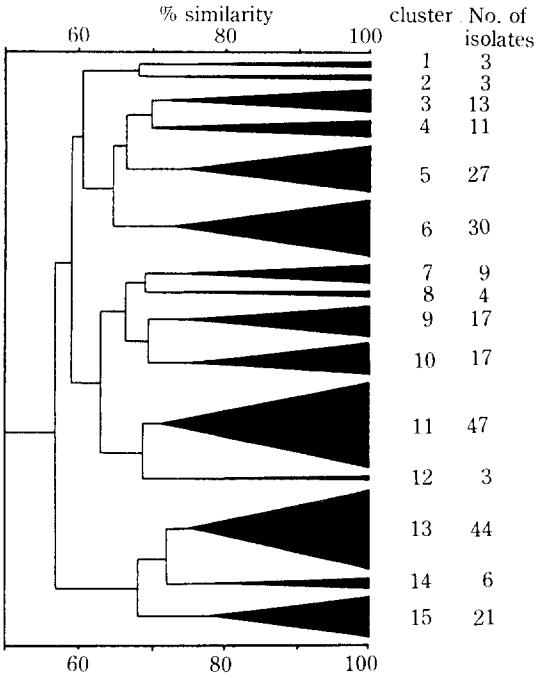

Fig. 5. *Simplified dendrogram of percent similarity showing relationships among all isolates from St. 4.*

5, 6, 8, 12 of St. 2, cluster 11 of St. 3, cluster 13 of St. 4). These strains resembled non-motile strains of *Alcaligenes* (Bonde, 1977). This group was predominant at St. 1 and 2.

4. Gram-negative, non-motile, non-pigmented, rods-coccobacilli (cluster 2 of St. 1, clusters 2, 3 of St. 2, cluster 2 of St. 3). The morphological and metabolic features of these organisms resembled those of *Acinetobacter-Moraxella* group (Thorney, 1967; Bøvre and Juni, 1984). The genera *Acinetobacter* and *Moraxella* are distinguished by the oxidase test, the former being negative and the latter positive (Baumann *et al.*, 1968). Thus, the strains in cluster 2 of St. 1 and cluster 2 of St. 2 were similar to *Acinetobacter*, and the others were similar to *Moraxella*. These organisms were dominant at St. 3.

5. Gram-positive, non-motile, oxidase-negative, cocci (cluster 5 of St. 1, cluster 11 of St. 2, cluster 10 of St. 3, clusters 9, 10 of St. 4). These organisms were morphologically similar to members of family *Micrococcaceae* (Sneath *et al.*,

Table 3. *Features of selected characteristics of major clusters in bacterial populations isolated from each station of Naktong Estuary*

station	1								2														3					4															
cluster	1	2	5	6	7	10	11	12	1	2	3	4	5	6	7	8	11	12	13	14	17	1	2	3	5	6	9	10	11	12	13	15	16	3	4	5	6	9	10	11	13	15
No. of isolates	34	36	11	27	41	23	9	11	36	16	9	8	30	15	15	17	15	29	22	13	8	11	8	20	61	15	8	20	12	34	25	7	15	13	11	27	30	17	17	47	44	21
character																																											
Gram stain	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	+	−	−	−
cell morphology	r	d	c	r	r	r	r	r	r	d	d	r	r	r	r	r	c	r	r	r	r	d	d	r	d	r	r	c	r	r	r	r	r	r	r	r	r	c	c	r	r	r
motility	d	−	−	−	−	+	+	d	−	−	−	−	−	−	d	−	−	−	d	+	−	d	−	d	−	+	−	−	−	d	+	−	−	+	*d*	−	+	−	−	+	−	d
pigment	−	−	d	+	−	−	−	−	−	−	−	d	−	−	−	−	d	−	d	−	−	−	−	d	−	−	+	−	−	−	d	−	−	−	−	−	−	d	−	−	−	d
OF test	−	−	−	−	−	F	F	F	−	−	f	−	−	−	−	−	f	−	−	F	F	−	F	F	−	−	−	−	−	F	f	F	F	−	−	F	F	−	F	O	−	o
oxidase	+	d	d	+	+	+	d	+	+	d	+	+	+	+	+	+	d	+	+	+	−	+	+	+	+	+	d	−	+	+	+	+	+	+	+	+	+	d	−	+	+	+
catalase	+	+	+	+	+	+	+	+	+	d	+	d	+	+	+	+	+	+	+	+	−	+	+	+	+	+	d	+	+	+	+	+	+	d	+	d	+	+	+	+	+	+
indole	−	−	−	−	−	d	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
MR	−	−	−	−	−	d	+	d	−	−	−	d	−	−	−	+	+	−	d	+	−	−	−	−	−	+	−	−	−	d	−	f	F	F	−	−	F	F	−	F	O	−	o
VP	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	d	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	
degradation of																																											
starch	−	−	−	d	−	d	d	−	−	−	−	+	−	+	−	−	+	−	−	−	−	−	−	d	−	+	+	−	+	+	d	−	+	d	+	+	+	d	−	−	−	−
tween 80	−	+	−	+	−	+	+	−	−	d	−	−	+	−	−	−	+	+	d	−	−	d	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
gelatine	−	−	d	d	d	+	d	−	−	−	−	−	−	+	−	−	+	+	d	d	−	d	−	d	−	+	+	−	+	+	−	+	+	d	+	+	+	+	*d*	−	−	+
growth at/in																																											
4 °C	d	+	−	−	−	+	+	+	+	+	d	−	d	d	d	d	+	+	+	+	d	+	d	+	−	+	d	+	+	+	d	−	+	+	+	+	+	+	d	+	−	d
37 °C	+	+	+	−	d	+	+	+	+	+	−	d	−	+	+	d	+	+	+	d	+	+	+	+	−	+	−	+	d	−	+	−	d	+	+	d	d	+	+	+	−	+
44 °C	−	−	d	−	−	−	+	d	−	d	−	d	−	d	−	−	d	−	d	−	+	d	+	−	−	+	−	−	−	−	d	−	−	−	−	−	−	d	+	d	−	d
pH 4.5	−	−	−	−	−	d	+	+	−	d	−	−	−	−	−	−	+	d	+	+	d	−	−	−	d	−	d	d	d	−	−	−	−	+	−	d	−	+	+	−	−	
pH 9.5	+	+	+	−	d	+	+	+	+	+	−	d	−	−	d	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	d	+	+	+	+
NaCl 0%	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	*d*	+	+	−	−	−	+	−	+	+	+	+	+	−	+
NaCl 1.5 %	+	+	+	−	−	+	+	+	+	+	d	−	d	d	+	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
NaCl 3.0%	−	−	+	−	−	d	+	+	+	d	−	−	−	−	+	d	+	+	+	+	+	+	+	+	+	+	d	+	+	+	+	d	+	+	+	+	+	+	+	d	+	
NaCl 5.0%	−	−	+	−	−	−	d	d	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	−	+	−	d	+	+	+	−	+	d	d	+	+	+	+	−	+	
NaCl 10.0%	−	−	+	−	−	−	d	−	−	−	−	−	−	−	−	+	−	−	−	−	−	d	d	−	−	d	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−
KCl	+	+	d	d	d	+	+	+	d	+	+	+	d	−	d	−	+	+	+	+	+	+	d	+	−	+	−	+	+	+	+	−	−	−	+	−	+	+	+	+	−	+
nitrate reduction	d	d	−	−	d	d	−	d	+	−	−	d	d	−	−	−	+	+	d	−	+	+	+	d	−	−	−	−	d	+	−	−	+	d	+	d	+	−	+	+	−	−

+ ≥ 70%; − ≤ 30%; 30% < d < 70%; f, slightly fermentative; o, slightly oxidative

1986). According to present classification schemes (Kloos *et al.*, 1975; LeChevallier, 1980), fermentative cocci are assigned to the genus *Staphylococcus* and non-fermentative ones to *Micrococcus*. Thus, the strains in cluster 11 of St. 2 and cluster 10 of St. 4 were identified as *Staphylococcus* and the others as *Micrococcus*.

6. Gram-negative, non-motile, pigmented (cluster 6 of St. 1, clusters 4, 13 of St. 2, cluster 9 of St. 3). According to the taxonomic keys of Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984), strains in these clusters were identified as genus *Flavobacterium*. This genus is not clearly classified, as defined primarily on pigment production (Gibson *et al.*, 1977).

7. Gram-negative, fermentative, oxidase-negative, rods (cluster 17 of St. 2, clusters 15, 16 of St. 3, cluster 5 of St. 4). According to the taxonomic key of Bonde (1977), these organisms were classified into the family *Enterobacteriaceae*.

DISCUSSION

Most of dominant groups were Gram-negative and rod-shaped bacteria, and could be identified as genera *Pseudomonas, Aeromonas, Vibrio, Acinetobacter, Moraxella, Alcaligenes, Flavobacterium,* and families *Enterobacteriaceae* and *Micrococcaceae*.

Pseudomonas, which have been isolated from a wide range of habitats within the aquatic environment (Murchelano and Brown, 1970; Austin *et al.*, 1979; Grimes *et al.*, 1984; Kuroda and Sakamoto, 1986) were also isolated in moderately high numbers from Naktong Estuary. Since genus *Pseudomonas* is subdivided into very numerous species which are very distinctive in metabolic activities and ecological habitats (Sands *et al.*, 1970; Palleroni, 1981; DeLey and DeVos, 1984), it would be useful to assess the ability of these strains to degrade recalcitrant and to measure their resistance to toxic materials in the study of ecological significance of *Pseudomonas*. In case of *P. aeruginosa*, it has often been used as an indicator organism of water pollution (Dutka and Kwan, 1977; Vicente *et al.*, 1986).

Vibrio is commonly found as a dominant genus is estuarine and marine environments, and it requires NaCl to grow (Murchelano and Brown, 1970; Austin *et al.*, 1979; Hauxhurst *et al.*, 1980; Grimes *et al.*, 1984; Hunter *et al.*, 1986). But some species of *Vibrio* are often found in freshwater (Sarkar *et al.*, 1983; Bockemühl *et al.*, 1986), and the densities increase below 5‰ of salinity in coastal areas (Seidler and Evans, 1984). Although *Aeromonas* is often found as a dominant genus in freshwater (Rippey and Cabelli, 1980; Burke *et al.*, 1984; Quinn *et al.*, 1985) and the true *Aeromonas* species has never been isolated from the marine environment (Simidu and Kaneko, 1973), there are some articles that *Aeromonas* is a normal floral constituent of brackish and marine habitats (Kaper *et al.*, 1981; Peele *et al.*, 1981). The density of *A. hydrophila* is used as a relative eutrophic index (Rippey and Cabelli, 1980). Seidler *et al.* (1980) and Kaper *et al.*(1981) have found that an inverse correlation exists between dissolved oxygen and the incidence of *Aeromonas* spp., and suggest that high density of *A. hydrophila* may be an indicator of eutrophic or nutrient-rich conditions. The densities of *A. hydrophila* also showed a negative correlation with DO and positive one with nutrient loadings in Naktong Estuary (Jeon, 1988).

Acinetobacter-Moraxella group readily isolated from marine habitats (Austin *et al.*, 1977; Hauxhurst *et al.*, 1980; Grimes *et al.*, 1984) was also found. But the characterization of this group is preliminarily based on the morphological features by most investigators. *Acinetobacter-Moraxella* groups isolated from aquatic environment are not sufficiently well studied to be readily seperated from the other Gram-negative, nonmotile, nonpigmented bacteria (Gibson *et al.*, 1977).

There were also problems to classify some clusters into genera *Alcaligenes* and *Achromobacter*. According to Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984), genus *Alcaligenes* is motile, while Thornley (1967) and Bonde (1977) reported nonmotile strains of *Alcaligenes*. According to Thornley (1967), most strains of *Achromobacter* have been identified as genus *Acinetobacter*.

Genus *Flavobacterium* is also preliminarily characterized by single distinctive feature, the reliance on yellow-orange pigmentation. Thus, this genus has been object of controversy in recent years, and several proportions have been made for rearrangement and differentiation (McMeekin *et al.*, 1972; Gibson *et al.*, 1977; Poen *et al.*, 1984). The ecological habitat of *Flavobacterium* is broad (Weeks, 1981), and this genus is found in many aquatic ecosystems (Murchelano and Brown, 1970; Buckley *et al.*, 1976; Hauxhurst *et al.*, 1980; Kuroda and Sakamoto, 1986).

There has been reported the existence of families *Micrococcaceae* and *Enterobacteriaceae* in marine habitats (Anderson, 1962; Murchelano and Brown, 1970; Gunn and Colwell, 1983; Hunter *et al.*, 1986), and Grimes *et al.* (1984) suggest that staphylococci can be an active component of the marine microbial community. But in this study, the densities of *Micrococcaceae* and *Enterobacteriaceae* were very low in ordinary times and rather concentrated in spring and summer. Therefore, it could be thought that the two families were allochthonous groups in Naktong Estuary and inflowed from terrestrial habitat due to rain. Heavy rains are known to increase allochthonous bacteria in lakes (Collins, 1960).

적 요

수리학적 방법을 이용하여 낙동강 하구에서 분리한 종속영양세균을 분류하였다. 70%의 유사도로 나눈 결과, 정점 1에서 분리한 균주는 14 clusters로, 정점 2에서 분리한 균주는 17 clusters로, 정점 3에서 분리한 균주는 17 clusters로, 정점 4에서 분리한 균주는 15 clusters로 구분되었다. 각 cluster의 주요 생리학적 및 형태학적 특성으로 분류한 결과, 정점 1에서의 우점종은 *Aeromonas, Vibrio, Alcaligenes*로, 정점 2에서는 *Alcaligenes*로, 정점 3에서는 *Aeromonas, Vibrio, Moraxella-Acinetobacter* group으로, 정점 4에서는 *Pseudomonas*로 판명되었다.

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REFERENCES

1. Anderson, J.I.W., 1962. Studies of micrococci isolated from the North Sea. *J. Appl. Bacteriol.* **25:** 362-368.
2. Austin, B., S. Garges, B. Conrad, E.E. Harding, R.R. Colwell, U. Simidu, and N. Taga, 1979. Comparative study of the aerobic, heterotrophic bacterial flora of Chesapeake Bay and Tokyo Bay. *Appl. Environ. Microbiol.* **37:** 704-714.
3. Baumann, P., M. Doudoroff, and R.Y. Stainer, 1968. A study of the *Moraxella* group. II. Oxidase-negative species (genus *Acinetobacter*). *J. Bacteriol.* **95:** 1520-1541.
4. Bockemuhl, J., K. Roch, B. Wohlers, V. Alesic, S. Alesic, and R. Wokatsch, 1986. Seasonal distribution of facultatively enteropathogenic vibrios (Vibrio cholera, Vibrio mimicus, Vibrio parahaemolyticus) in the freshwater of the Elbe River at Hamburg. *J. Appl. Bacteriol.* **60:** 435-442.
5. Bonde, G.J., 1977. Bacterial indicator of water pollution. In: Advances in aquatic microbiology. Vol. I. pp. 273-364. ed. by M.R. Droop and H.W. Jannasch. Academic Press Inc. London.
6. Bøvre, K. and E. Juni, 1984. Genus *Moraxella* and *Acinetobacter*. In: Bergey's manual of systematic bacteriology. Vol. I. pp. 296-307. ed. by N.R. Krieg and J.G. Holt. Williams and Wilkins. Baltimore.
7. Buckley, E.N., R.B. Joans, and F.K. Pfaender, 1976. Characterization of microbial isolates from an estuarine ecosystem: relationship of hydrocarbon utilization to ambient hydrocarbon concentrations. *Appl. Environ. Microbiol.* **32:** 232-237.

8. Burke, V., J. Robinson, M. Gracey, D. Peterson, and K. Patridge, 1984. Isolation of *Aeromonas hydrophila* from a metropolitan water supply: seasonal correlation with clinical isolates. *Appl. Environ. Microbiol.* **48:** 361-366.
9. Collins, V.G., 1960. The distribution and ecology of gram-negative organisms other than *Enterobacteriaceae* in lakes. *J. Appl. Bacteriol.* **23:** 510-514.
10. Colwell, R.R. and J.D. Walker, 1977. Ecological aspects of microbial degradation of petroleum in the marine environment. *Crit. Rev. Microbiol.* **5:** 423-445.
11. DeLey, J. and P. DeVos, 1984. Biological and clinical aspects of *Pseudomonas*. Antonie van Leeuwenhoek. **50:** 281-303.
12. Dutka, B.J. and K.K. Kwan, 1977. Confirmation of the single-step membrane filtration procedure for estimating *Pseudomonas aeruginosa* densities in water. *Appl. Environ. Microbiol.* **33:** 240-245.
13. Gibson, D.H., M.S. Hendrie, N.C. Houston, and A. Hobbs, 1977. The identification of some Gram negative heterotrophic aquatic bacteria. In: Aquatic Microbiology. pp. 135-160. ed. by F.A. Skinner and J.M. Shewann. Academic Press. London.
14. Grimes, D.J., F.L. Singleton, and R.R. Colwell, 1984. Allogenic succesion of marine bacterial communities in response to pharmaceutical waste. *J. Appl. Bacteriol.* **57:** 247-261.
15. Gunn, B.A. and R.R. Colwell, 1983. Numerical taxonomy of staphylococci isolated from the marine environment. *Int. J. Syst. Bacteriol.* **33:** 751-759.
16. Hauxhurst, J.D., M.I. Krichevsky, and R.M. Atlas, 1980. Numerical taxonomy of bacteria from the Gulf of Alaska. *J. Gen. Microbiol.* **120:** 131-148.
17. 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.
18. Jeon, D.Y., 1988. Distribution and characteristics of *Aeromonas* spp. isolated from Naktong Estuary. MS thesis. Seoul National Univ.
19. Kaper, J.B., H. Lockman, R.R. Colwell, and S.W. Joseph, 1981. *Aeromonas hydrophila:* ecology and toxigenicity of isolates from an estuary. *J. Appl. Bacteriol.* **50:** 359-377.
20. Kloos, W.E. and K.H. Schleifer, 1975. Isolation and characterization of staphylococci from human skin. II. Descriptions of four new species: *Staphylococcus warneri. S. capitis, S. hominis,* and *S. simulans. Int. J. Syst. Bacteriol.* **25:** 62-79.
21. Krieg, N.R. and J.G. Holt, 1984. Bergey's manual of systematic bacteriology. Vol. I. Williams and Wilkins. Baltimore.
22. Kuroda, N. and M. Sakamoto, 1986. A study on the structure of the heterotrophic bacterial community in the lake Suwa by colony grouping method. *Jpn. J. Limnol.* **47:** 229-237.
23. 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.
24. LeChevallier, M.W., R.J. Seidler, and T.M. Evans, 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *Appl. Environ. Microbiol.* **40:** 922-930.
25. McMeekin, T.A., D.B. Stewart, and J.G. Murray, 1972. The Adansonian taxonomy and the deoxyribonucleic acid base composition of some Gram negative, yellow pigmented rods. *J. Appl. Bacteriol.* **35:** 129-137.
26. Murchelano, R.A. and C. Brown, 1970. Heterotrophic bacteria in Long Island Sound. *Mar. Biol.* **7:** 1-6.
27. Palleroni, N.J., 1981. Introduction to the family *Pseudomonadaceae.* In: The Prokaryotes. pp. 655-665. ed. by M.P. Starr *et al.* Springer-Verlag. Berlin.
28. Peele, E.R., F.L. Singleton, J.W. Deming, B. Cavari, and R.R. Colwell, 1981. Effects of

pharmaceutical wastes on microbial populations in surface waters at the Puerto Rico dump site in the Atlantic Ocean. *Appl. Environ. Microbiol.* **41:** 873-879.

29. Poen, E., M. Aufderheide, H. Diekmann, and R.M. Kroppenstedt, 1984. Taxonomic studies on filamentous bacteria from sewage belong to the *Flavobacterium-Cytophaga* complex. *Arch. Microbiol.* **137:** 295-301.
30. Quinn, J.P., M.B. Gillan, and H. McGrogan, 1985. The planktonic and benthic bacterial populations of Lough Neagh. *J. Appl. Bacteriol.* **58:** 87-93.
31. Rheinheimer, G., 1977. Microbial ecology of a brackish water environment. Springer-Verlag. Berlin.
32. Rippey, S.R. and V.J. Cabelli, 1980. Occurence of *A. hydrophila* in limnetic environments: relationship of the organism to trophic state. *Microb. Ecol.* **6:** 45-54.
33. Sands, D.C., M.N. Schroth, and D.C. Hildebrand, 1970. Taxonomy of phytopathogenic pseudomonads. *J. Bacteriol.* **101:** 9-23.
34. Sarker, B.L., G. Balakrish, B.K. Sircar, and S.C. Pal, 1983. Incidence and level of *V. parahaemolyticus* associated with freshwater plankton. *Appl. Environ. Microbiol.* **46:** 288-290.
35. Seidler, R.J. and T.M. Evans, 1984. Computer-associated analysis of Vibrio field data: four coastal areas. In: Vibrios in the environment. pp. 411-425. ed. by R.R. Colwell. John Wiley & Sons. New York.
36. Seidler, R.J., D.A. Allen, H. Lockmann, R.R. Colwell, S.W. Joseph, and O.P. Daily, 1980. Isolation, enumeration and characterization of *Aeromonas* from polluted waters used for diving operations. *Appl. Environ. Microbiol.* **39:** 1010-1018.
37. Shewann, J.M., G. Hobbs, and W. Hodgekiss, 1960. A determinative scheme for the identification of certain genera of Gram-negative bacteria, with special reference to the *Pseudomonadaceae. J. Appl. Bacteriol.* **23:** 379-390.
38. Simidu, U. and E. Kaneko, 1973. A numerical taxonomy of *Vibrio* and *Aeromonas* from natural and diseased marine fish. *Bull. Jpn. Soc. Sci. Fish.* **39:** 689-703.
39. Sneath, P.H. and R.R. Sokal, 1973. Numerical taxonomy, the principles and practice of numerical classification, W.H. Freeman and Co. San Francisco.
40. Sneath, P.H., N.S. Mair, M.E. Sharpe, and J.G. Holt, 1986. Bergey's manual of systematic bacteriology. Vol. II. Williams and Wilkins.
41. Star, M.P., H. Stolp, H.G. Truper, A. Balows, and H.G. Schlegel, 1981. The Prokaryotes. Springer-Verlag. Berlin.
42. Tagger, S., A. Bianchi, M. Julliard, J. LePetit, and B. Roux, 1983. Effect of microbial seeding of crude oil in seawater in a model system. *Mar. Biol.* **78:** 13-20.
43. Thornley, M.J., 1967. A taxonomic study of *Acinetobacter* and related genera. *J. Gen. Microbiol.* **49:** 211-257.
44. Vicente, A., J.J. Borrego, F. Arrabal, and P. Romero, 1986. Comparative study of selective media for enumeration of *Pseudomonas* aeruginosa from water by membrane filtration. *Appl. Environ. Microbiol.* **51:** 832-840.
45. Weeks, O.B., 1981. The genus *Flavobacterium.* In: The Prokaryotes. ed. by M.P. Starr *et al.* Springer-Verlag. Berlin.

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