Seasonal Analysis of Heterotrophic Bacterial Community in Lake Soyang

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소양호 세균 군집의 계절적 분석

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ABSTRACT: The numerical taxonomy of heterotrophic bacterial community in Lake Soyang was analysed. 95, 115, 88 and 75 strains which were isolated at each season from spring in 1987 to winter in 1988 were clustered by single matching coefficient. The diversity indices (H') were in the range of 0.511-1.684, and the community was most diverse in spring. The seasonal variation of generic composition was significant. Of the dominant genera, *Acinetobacter*, *Pseudomonas*, and *Flavobacterium* were representative.

KEY WORDS □ Heterotrophic bacteria, cluster analysis, diversity index, freshwater, Lake Soyang

The saprophytic bacteria form colonies on the relatively high nutrient concentration agar medium, and they comprise of very small portions of total bacterial number counted by acridine orange direct counts (AODC) (Rheinheimer, 1985). The saprophytic bacteria are the most active heterotrophic bacterial group, and thus they represent heterotrophic bacteria.

The composition and the fluctuation of the communities of heterotrophic bacteria are important to appreciate the role of bacterial populations and the interactions between bacterial communities and their environments in natural ecosystems since all bacteria can not be cultivated in a medium. The change in the structure of the communities can be elucidated by species diversity, which is a measure of the species composition of an ecosystem in terms of the number and relative abundances of the species (Atlas, 1984). Because of the difficulty in identifying bacterial species based on cell and colony morphology, bacterial classification should be done with the aid of physiological and biochemical tests.

The distribution of heterotrophic bacteria and their community structure are affected by various environmental factors such as temperature, nutrients, etc. The fluctuation of species diversity are related to the environmental fluctuation, and communities in constant environments shoud be more diverse than those in fluctuating environments (Rashit and Bazin, 1987). The diversity can be expressed by Shannon-Weaver index (H', Shannon and Weaver, 1949), and H' has its minimum value (0) when a community is composed of only one species.

Lake Soyang, the largest man-made lake in Korea, lies in the center of Korean Peninsula and upstream of Han River flowing through Seoul City. Because of the effects of the continental climate and monsoon, the difference of water temperature between summer and winter is more than 20°C, and the precipitation is concentrated on the rainy season of summer. The deepest site of the lake reaches more than 100 m, and thermal stratification occurs in summer. In this dentritic type lake, blooming of *Peridinium* spp. appears as red tide

intermittently throughout the year in the tributary stream. This environmental factors influences on the distribution of heterotrophic bacteria temporally and spatially, and the number of saprophytic bacteria fluctuates very severely.

In this study, the numerical analysis of bacterial communities was done in comparison with phylogenetic classification. The heterotrophic bacteria from Lake Soyang were isolated through four seasons from the spring in 1987 to the winter in 1988.

MATERIALS AND METHODS

Colonies formed on ZoBell 2216E agar plate displaced with distilled water were isolated randomly and transferred to the new plates until pure isolates were obtained. About 100 strains were isolated in each season, and 95, 115, 88, 75 strains were analysed for 47 characters isolated in April, July, and September in 1987 and February in 1988, respectively. The characters used in this study refer to morphological, physiological, and biochemical aspects (Table 1). Cell shape was discriminated as rod, coccus, or sprial and observed in parallel with Gram-stain. Colony morphology was divided into 6 items and the detail characters are represented in Table 2. Characters which show more than one property were coded as multistate characters. Colony morphology were described original plate for saprophyte counting. In the test for physiological characters, 2216E agar plate media were modified according to the pH and salt concentration, but broth medium was used for pH 4. Gram-stain, motility, biochemical characters, and substrate utilization were tested according to Smibert and Krieg (1981) and MacFaddin (1980). The carbohydrate utilization were tested according to MacFaddin (1980), but multi-well tubes were used, and the concentration of salicin was 0.5% and others were 1.0%.

Cluster analysis was performed separately for each season, and the similarity index was calculated as the percentage of the number of characters shared by two strains to the total characters tested. The culerstring method follows the single linkage clustring (Sokal and Sneath, 1973). The computer process of clustering was done with VAX 11 Computer System of Seoul National University. The program for clustering was designed with FORTRAN language.

Table 1. *Tests used for the character analysis of bacterial isolates*

Morphology
Cell shape, Gram-stain, Colony Morphology, Motility
Physiological Characters
Growth at 4, 37, 44 °C
Growth at pH 4, 10
Growth a 1.6, 3.5, 10% NaCl, and 3.5% KCl
Biochemical Characters
Aesculin hydrolysis, Catalase production, Citrate test, Gas production, Indole formation, Methyl-red test, Nitrate reduction, O/129 sensitivity, Oxidation/Fermentation test, Voges-Proskauer test, Oxidase production, Urease production
Substrate Utilization
Arginine hydrolysis, Casein hydrolysis, Gelatin liquefaction, Starch hydrolysis
Carbohydate Fermentation
Adonitol, Arabinose, Dulcitol, Fructose, Galactose, Inocitol, Lactose, Mannitol, Raffinose, Rhamnose, Sorbitol, Sucrose, Salicin

Table 2. *Characters for colony morphology*

Form
Circular, Filamentous, Irregular, Spindle, Rhizoid
Elevation
Flat, Raised, Convex, Pulvinate, Umbonate
Opacity
Transparent, Translucent, Opaque
Size ϕ
< 2 mm, 2-5 mm, > 5 mm
Pigmentation
Yellow, Milk, Skin, Red, Violet, Orange, White, Brown
Texture
Dry, Viscous

By using determination schemes (Shewan *et al.*, 1960; Krieg and Holt, 1984) the bacterial strains were classified to genus level, and the seasonal variation of generic composition was also analysed.

RESULTS

The numbers of clusters for each season were highly variable and distributed in the range of 10-52 (Table 3). The dendrograms for each season

Table 3. *The data of numerical taxonomic analysis of the heterotrophic bacterial strains from Lake Soyang at 80% similarity level.*

Sampling time	Number of strains	Number of clusters	Diversity index (H')
Apr., 1987	95	52	1.684
Jul.	115	10	0.511
Sep.	88	27	1.299
Feb., 1988	75	22	1.020

are represented in Fig. 1, 2, 3 and 4. The diversity index (H') decreased significantly in summer and increased again in fall. And H' was decreased in winter again.

28 strains isolated in spring were not grouped, and this is 29.5% of total 95 strains. The number of ungrouped strains was 3 (3.6%) in summer. Both the number of ungrouped strains in fall and winter were 8, and the percentage were 9.1% and 10.7%, respecively. The main clusters of each season and the characters of strains belonged to the clusters are shown in Table 4-6 and 7. During the year, majority of bacterial isolates were Gram negative and rod type (more than 85%), but in spring and winter Gram positive bacteria slightly increased. More than 70% of the isolates in major clusters of summer had motility, but in other seasons most of the isolates did not motile. In general feaures, catalase-positive, indole-negative, MR-negative, starch-negative, 44°C-negative, pH 4-negative, pH 10-positive, and 10% NaCl-negative were the characteristics of majority of the isolates. In spring and winter, the nitrate reduction was not frequent character among the members of the major clusters.

The seasonal variation of generic composition of heterotrophic bacterial communities is shown in Table 8. The bacterial isolates were classified ten-

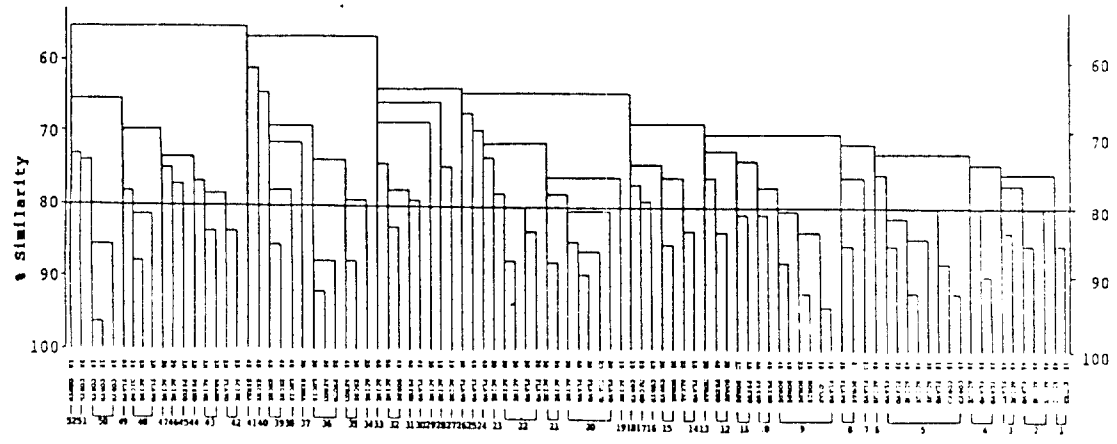

Fig. 1. *Dendrogram of bacterial strains isolated from Lake Soyang in April, 1987 at 80% similarity level.*

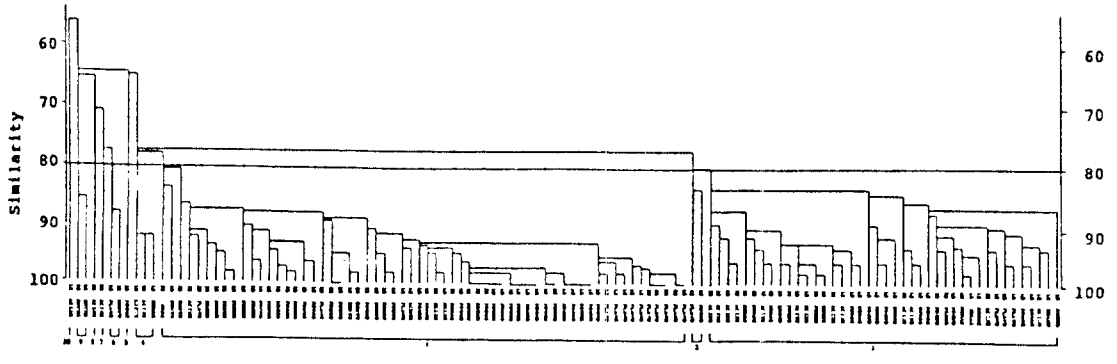

Fig. 2. *Dendrogram of bacterial strains from Lake Soyang in July, 198. at 80% similarity level.*

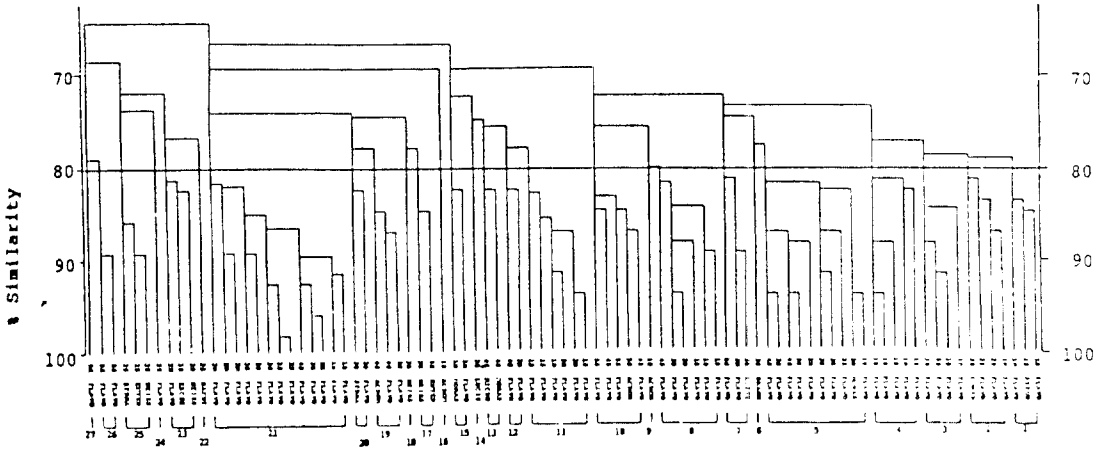

Fig. 3. *Denrogram of bacterial strains isolated from Lake Soyang in September, 1987 at 80% similarity level.*

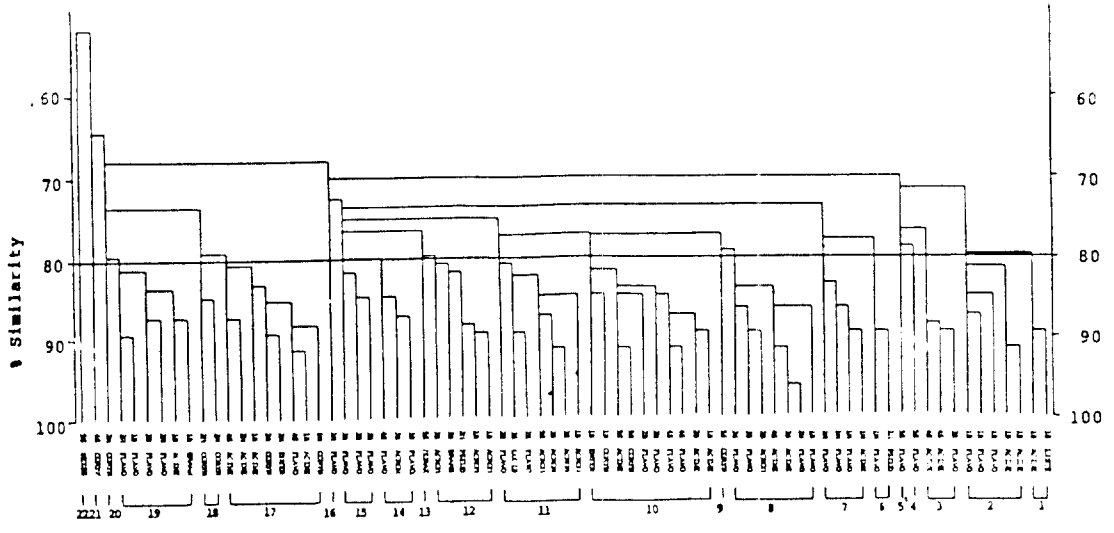

Fig. 4. *Dendrogram of bacterial isolated from Lake Soyang in February, 1988 at 80% similarity level.*

tatively as 18 genera. In spring the dominant genera were *Acinetobacter* (27%), *Flavobacterium* (26%), and *Corynebacterium* (11%). In summer *Pseudomonas* (42%), *Acinetobacter* (17%), *Flavobacterium* (16%), and *Bordetella* (12%) were dominant genera. The predominant genus in autumn was *Flavobacterium* (74%). The generic composition in winter was similar to that of spring, and *Flavobacterium, Acinetobacter, Aeromonas, Corynebacterium* were dominant genera.

DISCUSSION

The aboundance of bacteria is in inverse proportion to the diversity of bacterial community. The communities under the stress of environmental factors such as temperature or organic matters show high diversity since one species cannot predominate (Atlas, 1984). In Lake Soyang low water temperature and low concentration of organic matters in spring limited the exessive growth of certain species, but increased temperature and higher concentration of organic matters in summer permitted the overgrowth of well-adapted species. The number of hetero bacteria in summer was 100-fold of that of winter (unpublished data).

The diversity of bacterial community in

Table 4. *Features of selected characteristics of major clusters in bacterial communities isolated from Lake Soyang in April, 1987*

Cluster	5	9	20
No. of members	8	6	5
Character			
Gram stain	−	− −	− −
cell morphology	RR	RR	RR
motility	− −		−
O/F test	− −	− −	− −
oxidase	− −		−
catalase	+ +	+ +	+ +
indole	− −	− −	− −
MR	− −	− −	− −
Degradation of			
starch	− −	− −	− −
gelatine	− −		
Growth in/at			
4 °C	+	+ +	
37 °C	+ +	+ +	+ +
44 °C	− −	− −	− −
pH 4	− −	− −	− −
pH 10	+ +	+ +	+
NaCl 1.6%	+ +	+ +	+ +
3.5%	+ +	+ +	+
10.0%	− −	− −	− −
KCl 1.6%	+ +	+ +	+ +
Nitrate reduction	+ +		− −

+ +/− −: +/− reaction in more than 85% of members; +/−: +/− reaction in more than 70% and less than 85% of members.
R: rod.

Table 5. *Features of selected characteristics of major clusters in bacterial communities from Lake Soyang in July, 1987*

Cluster	1	2
No. of members	41	59
Characters		
Gram stain	− −	− −
cell morphology	RR	RR
motility	+	+
O/F test	− −	OO[a]
oxidase	+ +	+ +
catalase	+ +	+ +
indole	− −	− −
MR	− −	− −
Degradation of		
starch	− −	− −
gelatine		+ +
Growyh in/at		
4 °C	− −	− −
37 °C	+ +	+ +
44 °C	+ +	+ +
pH 4	−	− −
pH 10	+ +	+ +
NaCl 1.6%	+ +	+ +
3.5%	+ +	+ +
10.0%	− −	− −
KCl 1.6%	+ +	+ +
Nitrate reduction		

a. O: Oxidative

September, when phytoplankton blooming occurred, was low as compared with that of April. It suggests that increased organic matter produced by pytoplankton stimulated and selected specific bacterial groups. Phytoplankton stimulate growth of bacteria by producing organic nutrients, but also inhibit by producing biologically active compounds such as antibiotics (Metting and Pyne, 1986; Pesando and Caram, 1984). Thus by these two effects, mass production of phytoplankton in blooming selects bacterial community.

In winter the low water temperature (4-5°C) might act as an environmental stress, and hinder the bacterial growth. And bacterial diversity was decreased to H' = 1.02. The environmental stress alters the composition of bacterial communities, and in Lake Soyang physicochemical factors such as temperature and dissolved oxygen play an important role in determining of the abundance and diversity of bacterial community (Atlas, 1984).

The diversity index of bacterial communities in Lake Soyang was in the range of 0.511-1.684, and these values were very small as compared with that of Nakdong River Estuary, which showed the range of 1.86-3.31 (Kwon, 1987). But in comparison with the results of experiments by Gehlen *et al.* (1985) in ground water of low nutrient (H' = 0.3-0.7) and surface water from an oligotrophic artificial lake (H' > 2.5) Lake Soyang as the relatively

Table 6. *Features of selected characteristics of major clusters in bacterial communities isolated from Lake Soyang in September, 1987*

Cluster	5	8	11	21
No. of member	10	6	6	13
Characters				
Gram stain	− −	− −	− −	− −
cell morphology	RR	RR	RR	RR
motility	− −	− −	− −	− −
O/F test	− −		−	FF[a]
oxidase	−	+ +		− −
catalase	+ +	+ +	+ +	+ +
indole		− −	− −	− −
MR	−	− −		− −
Degradation of				
starch	− −	− −	−	− −
gelatine	+ +	+ +	+	−
Growth in/at				
4 °C	− −	− −	− −	− −
37 °C	+ +	+ +	+ +	+ +
44 °C	+ +			+ +
pH 4	− −	− −	− −	− −
pH 10	+ +	+	+ +	+ +
NaCl 1.6%	+ +	+ +	+ +	+ +
3.5%		+	+	+ +
10.0%	− −	− −	− −	
KCl 1.6%	+ +	+ +	+ +	+ +
Nirate reduction		+ +	+	+ +

A: Fermentative.

Table 7. *Features of selected characteristics of major clusters in bacterial communities isolated from Lake Soyang in February, 1988*

Cluster	8	10	11	17
No. of member	7	10	7	8
Characters				
Gram stain	− −	−	− −	−
cell morphology	RR	RR	RR	RR
motility	− −	−	+	−
O/F test	− −	− −	− −	− −
oxidase	−	+	+ +	
catalase	+ +	+ +	+ +	+ +
indole	[11]	− −	− −	− −
MR	− −	− −	− −	− −
Degradation of				
starch	− −	− −	−	− −
gelatine	+ +	−	+ +	+ +
Growth in/at				
4 °C	− −	− −	− −	− −
37 °C	+ +	+ +	+ +	+ +
44 °C	+ +			
pH 4	− −	− −	− −	− −
pH 10	+ +	+	+ +	+ +
NaCl 1.6%	+ +	+ +	+ +	+ +
3.5%	[$$]	+ +	+	
10.0%	− −	− −	− −	
KCl 1.6%	+ +	+ +	+ +	+ +
Nirate reduction		+	+ +	− −

A: Fermentative.

Table 8. *Seasonal variation of generic composition of bacterial communities isolated from Lake Soyang during 1987-1988*[a].

Genera	Spring	Summer	Autumn	Winter
Acinetobacter	27	17	0	24
Aeromonas	3	1	5	12
Alcaligenes	1	0	0	0
Bordetella	9	12	3	0
Branhamella	1	2	1	3
Corynebacterium	11	4	1	12
Enterobacter	1	2	1	0
Escherichia	4	0	2	3
Flavobacterium	26	16	74	39
Hafnia	0	0	1	0
Listeria	0	2	1	1
Lucibacterium	2	0	1	1
Micrococcus	2	0	1	0
Moraxella	2	2	0	1
Neisseria	0	2	4	1
Photobacterium	1	0	0	0
Pseudomonas	6	42	1	3
Serratia	2	0	2	0

a. The proportion of each genus is expressed in the percentage to total isolates.

oligotrophic, but partially eutrophic lake in phytoplankton blooming season and in inflowing region showed the servere fluctuation of nutrient regime, and thus the diversity of bacterial community changed significantly.

In phylogenetic classification of bacterial isolates, the fluctuation of generic composition showed the interactions between bacterial communities and environmental factors. *Pseudomonas* was not dominant group in spring (6.3%), but comprise about half of the isolates in summer (42%). It suggests that *Pseudomonas* which has the metabolic potential of various organic compounds rapidly adapted to environmental change such as increase of temperature and organic matters. *Flavobacterium* occurred in high frequency during the year, and comprised 74% of total isolates in autumn when phytoplankton blooming was in descending. Also in the site of algal blooming the frequency of appearance was higher than other sites (data not shown). It suggests that *Flavobacterium* was selected by phytoplankton blooming. A significant correlation of a specific bacteria group to the phytoplankton blooms was recognized other research area (Bolter, 1977).

적 요

소양호 종속영양세균 군집에 대한 수리학적 분석을 수행하였다. 1987년 봄부터 1988년 겨울까지의 각 계절별로 분리된 95, 115, 88, 75개의 균주를 단순 유사도에 의해 통계학적으로 분석하였다. 다양성의 지표로서 H′은 0.511에서 1.684의 범위로 변화하였으며 봄철 세균 군집의 다양성이 가장 큰 것으로 나타났다. 각 속(genus)의 구성은 계절적 특성이 뚜렷하였으며, *Acinetobacter*, *Pseudomonas*, *Flavobacterium* 이 특징적으로 우점종을 이루었다.

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