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Dynamics of *in situ* Bacterial Community Structure in the Nak-Dong River

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For comparative analysis of the eubacterial community structure at 8 sampling sites throughout the Nak-Dong River, FISH (fluorescence *in situ* hybridization) method was employed. The total ratio of each determined eubacterial group such as α · β · γ-subclasses proteobacteria and *Cytophaga-Flavobacterium* (CF) group to total counts (DAPI) at each site varied 9.3-42.5% with the highest value at uppermost part. And each ratio of determined eubacterial groups reached mostly under 10% except that of CF group (23%) at uppermost part. Furthermore, compared to lower part, upper part represented unexpectedly higher proportions of γ-subclass proteobacteria comprised almost fast growing bacteria on degradable organics. Also the variations of ammonia-oxidizing bacteria ranged from 2.7×10^4 to 18.0×10^4 cells mL^{-1} with the lowest value in lower part and the highest value in mid part whereas those of nitrite-oxidizing bacteria varied $5.2-7.7 \times 10^4$ cells mL^{-1} without noticeable differences throughout the sites. Additionally, the ratio of nitrifying bacteria to total counts ranged from 1.0% to 13.6% with no differences between ammonia-oxidizing bacteria and nitrite-oxidizing bacteria. In conclusion, FISH method introduced in this study for monitoring, normally used for the quantitative analysis of bacteria, provided also good information on their environmental status in the Nak-Dong River.

Key words : FISH (fluorescence *in situ* hybridization), α · β · γ-subclasses proteobacteria, *Cytophaga-Flavobacterium* group, nitrifying bacteria, Nak-Dong River

INTRODUCTION

Until now, for environmental monitoring of the Nak-Dong River, one of the 4 main Korean Rivers with tremendous large watershed, most studies about this river have been restricted to their physico-chemical characteristics (Jeong and Cho, 2003), algae (Park *et al*., 2004) and fauna including zooplankton (Kim *et al*., 2003) and fishes (Kang *et al*., 2004). However the bacterial study was carried out only in its lower part (Kim and Lee, 1998), even though bacteria play an important role for recycling the nutrients in aquatic ecosystem. In order to analyze the bacterial community structure, the direct identification method, named fluorescent *in situ* hybridization (FISH), has been applied recently, because it was known that the bacterial populations isolated on culture medium could only be <1-10% of their whole populations (Amann *et al*., 1995). Moreover, this method would greatly facilitate their identification, independent of a time-consuming culture, and physiological and biochemical tests (Manz *et al*., 1992). Regarding the bacterial populations in an aquatic environment, most bacteria fall in gram-negative and many of them belong to the class Proteobacteria and *Cytophaga-Flavobacterium* (CF) group (Alfreider *et al*., 1996). Proteobacteria are subdivided into

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the α-, β-, γ- and δ-subclasses (Manz *et al.*, 1992). The objective of this study was to analyze the eubacterial community structure including nitrifying bacteria at 8 sampling sites from the upper part up to the lower part of the Nak-Dong River comparatively using FISH.

MATERIALS AND METHODS

The sampling sites with their description are shown in Fig. 1. and Table 1. Also the gene probes for the *in situ* identification of the eubacterial groups such as α-, β-, and γ-subclasses Proteobacteria and CF group with rRNA targeted oligonucleotides used in this study, and their performing methods were summarized in Table 2. The mean values for each group-specific bacteria and total counts were calculated from the counts of 15 randomly chosen fields using epifluorescent microscope (Zeiss, Axioplan), and the results were expressed in ratio (%) of the number of individual group-specific bacteria to the number of total bacteria (total counts) which were stained with DAPI (Glöckner *et al.*, 1999).

Fig. 1. Sampling sites in the Nak-Dong River and tributaries.

RESULTS AND DISCUSSION

Variations of total counts and eubacterial community structures throughout the Nak-Dong River

As shown in Fig. 2, the total counts varied in a narrow range of $6.5-35.0 \times 10^5$ cells mL^{-1}. Considering its 95% standard deviation, there was no differences depending on upper/mid/lower part of Nak-Dong River. Rather than, it could be subdivided into clean zone (1. Chong-Ryang), relatively clean zone (3. Sang-Pung, 4. Wae-Kwan) and polluted zone (2. An-Dong, 5. Ko-Ryung, 6. Nam-Ji, 7. Su-San, 8. Mul-Gum) in terms of water quality. So it revealed that the enumeration of total counts did not represent the *in situ* environmental situation precisely, especially in heavy polluted water. To the contrary, the results of *in situ* analysis of eubacterial community using FISH suggested that the ratio of eubacterial group to total counts in rather clean zones (sites: 1. Chong-Ryang, 42.5%; 3. Sang-Pung, 28.2%; 4. Wae-Kwan, 26.9%) was pretty higher than those in heavy polluted zones (sites: 2. An-Dong, 11.1%; 5. Ko-Ryung, 9.3%; 6. Nam-Ji, 9.7%; 7. Su-San, 10.0%; 8. Mul-Gum, 11.6%), even though it might be expected that a large amount of organic pollutants is most likely to be contained in such a heavy polluted lower part, as reported by others (Kim and Lee, 1998; Jeong and Cho, 2003; Kim *et al.*, 2003). Based on cell detectability of FISH resulted

Table 1. Descriptions of sampling sites and the temperature at each site.

Sites		Description of sampling site	Temp. (°C)
Upper	1. Chong-Ryang	Near forestry watershed land use	21.3
	2. An-Dong bridge	Near urban watershed land use	20.4
	3. Sang-Pung	Near arable watershed land use	21.2
Mid	4. Wae-Kwan	Before merging the Kum-Ho River	21.9
	5. Ko-Ryung	After merging the Kum-Ho River	23.2
Lower	6. Nam-Ji	After merging the Nam River	21.9
	7. Su-San	Before merging the Mil-Yang River	23.5
	8. Mul-Gum	Near industrial land use	22.1

Table 2. Oligonucleotide probes with their targeted organisms.

Probe	Sequence	Target site (*E. coli* rRNA positions)	Target organisms	Form−amide (%)	Reference
ALF1b	5′−CGTTCGTT CTGAGCCAG−3′	16S (19−35)	α−subclass of Proteobacteria	20	Manz *et al*., 1992
BET42a	5′−GCCTTCCC ACTTCGTTT−3′	23S (1027−1043)	β−subclass of Proteobacteria	35	Manz *et al*., 1992
GAM42a	5′−GCCTTCCC ACATCGTTT−3′	23S (1027−1043)	γ−subclass of Proteobacteria	35	Manz *et al*., 1992
CF319a	5′−TGGTCCGTG TCTCAGTAC−3′	16S (319−336)	*Cytophaga−Flavo-bacterium* group	15	Wagner *et al*., 1996
NSO190	5′−CGATCCCCT GCTTTTCTCC−3′	16S (190−208)	Ammonia oxidizing bacteria	55	Schramm *et al*., 1998
NIT3	5′−CCTGTGCTC CATGCTCCG−3′	16S (1030−1047)	Nitrite−oxidizing bacteria	40	Wagner *et al*., 1996

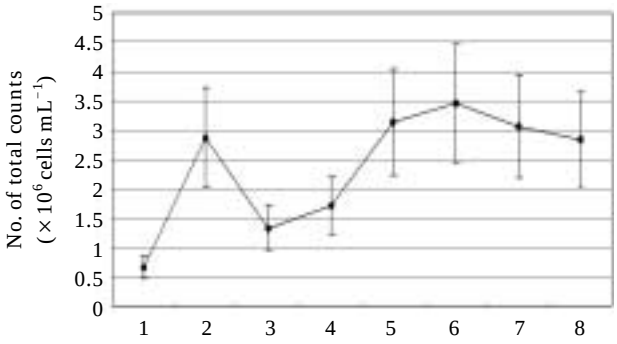

Fig. 2. Variations of total counts in the Nak−Dong River (1. Chong−Ryang, 2. An−Dong, 3. Sang−Pung, 4. Wae−Kwan, 5. Ko−Ryung, 6. Nam−Ji, 7. Su−San, 8. Mul−Gum, I: standard deviation).

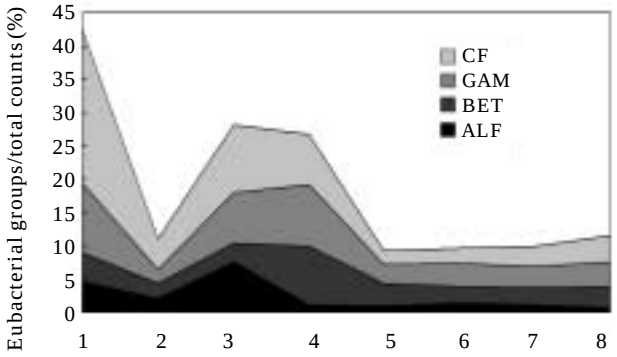

Fig. 3. Proportions of α−, β−, γ− proteobacterial groups and *Cytophaga−Flavobacterium* group detected by group−specific fluorescent probes to total counts stained with DAPI at each site (1. Chong−Ryang, 2. An−Dong, 3. Sang− Pung, 4. Wae−Kwan, 5. Ko−Ryung, 6. Nam−Ji, 7. Su−San, 8. Mul−Gum.) ALF = α−subclass, BET = β−subclass, GAM = γ−subclass, CF = *Cytophaga−Flavobacterium* group) of the Nak−Dong River.

from their cellular activity Hicks *et al*. (1992), it suggests that the bacterial decomposing activities in the lower part of Nak−Dong River, especially after merging the Gum−Ho River may be inhibited compared to those in the upper part.

Variations of nitrifying bacteria in the Nak−Dong River

Nitrification, the oxidation of ammonia to nitrate via nitrite is achieved by slow growing nitrifying bacteria (Wagner *et al*., 1996). When used gene probe, NSO190, it could be detected following ammonia−oxidizing bacteria; *Nitrosomonas* sp., *Nitrosococcus mobilis*, *Nitrosospira* sp. (Schramm *et al*., 1998) and they belong to β−subclass proteobacteria (Kowalchuk *et al*., 1999). NIT3 probe enabled the detection of *Nitrobacter* sp. including *Nitrobacter winogradsky*, *N. hamburgensis* and it belongs to α−subclass proteobacteria (Schramm *et al*., 1998). Similar to the results of group−specific eubacteria, ammonia−oxidizing bacteria were more abundant in rather clean zones (sites: 1. Chong−Ryang, 13.6%; 3. Sang−Pung, 12.8%; 4. Wae−Kwan, 9.5%) than in other heavy polluted zones (1.5−5.8%), that is, it ranged from 2.7×10^4 to 18.0×10^4 cells mL^{-1} with the lowest value in

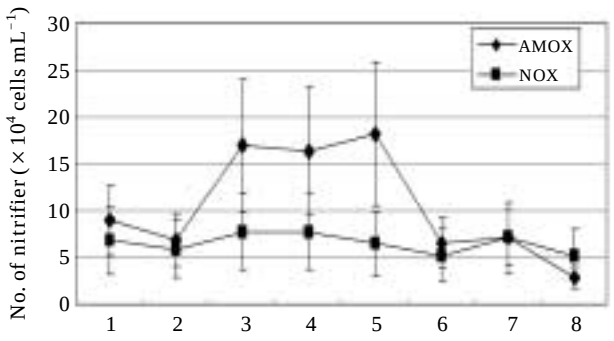

Fig. 4. The variations of ammonia-oxidizing bacteria (AMOX)and nitrite-oxidizing bacteria (NOX) at each site (1. Chong-Ryang, 2. An-Dong, 3. Sang-Pung, 4. Wae-Kwan, 5. Ko-Ryung, 6. Nam- Ji, 7. Su-San, 8. Mul-Gum.) of the Nak-Dong River (I: Standard Deviation).

lower part and the highest value in upper/mid part. However nitrite-oxidizing bacteria varied $5.2-7.7 \times 10^4$ cells mL^{-1} without noticeable differences throughout the sites, maybe due to the fact that its population size is influenced by environmental factors such as DO rather than temperature, nutrients (Schramm *et al*., 1998). Furthermore it is also possible that the other nitrite-oxidizer which were not detectable using NIT3 could be present. Additionally, the ratio of nitrifying bacteria to total counts ranged from 1.0% to 13.6% with no differences between ammonia-oxidizer and nitrite-oxidizer.

Although the FISH method using group-specific oligonucleotide probes provided information not on species level but on a rougher scale, it appears to be a useful tool for analyzing the status of water quality of natural environment, because the cell detection depends on their ribosome contents (Hicks *et al*., 1992).

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＜**국문적요**＞

낙동강에서의 세균군집구조의 역동성

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낙동강 전 수계 8개 정점에서 분자기법인 FISH (Fluorescent *in situ* Hybridization)법으로 세균군집구조를 비교분석하였다. Eubacteria에 속하는 α · β · γ-subclasses proteobacteria와 CF group 세균의 합이 총세균수에서 차지하는 비율이 9.3-42.5%에서 변화하였고 그 최고치는 최상류, 청량에서 나타났다. 각 세균그룹이 총세균수에서 차지하는 비율이 10% 미만이었으나 최상류에서의 CF 그룹이 총세균수에서 차지하는 비율은 23%이었다. 또한 유기물질을 분해해서 빠른 성장을 한다는 γ-subclasses proteobacteria 세균군이 예상과는 달리 유기오염정도가 높은 하류에 비해 상류에서 더 많이 검출되었다. 아울러 암모니아산화세균은 $2.7-18.0\times10^4$ cells mL^{-1}의 범위에서 변화하였고 하류에서 최저치를 그리고 최고치는 중류에서 보였다. 반면에 아질산산화세균의 경우, 전수계에 걸쳐 정점간의 별 차이 없이 $5.2-7.7\times10^4$ cells mL^{-1}에서 변화하였으며 그들이 총세균수에서 차지하는 비율은 두세균군간의 차이없이 1.0-13.6%에서 변화하였다. 결론적으로 FISH법은 통상적으로 세균군집의 정량적인 분석에 사용되지만 그 결과는 본 연구결과에서 보는 바와 같이 수계 환경의 현황에 관한 좋은 정보를 제공해주기도 한다.