Bacterial Community Composition and Activity in Urban Rivers in Thailand and Malaysia

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The phylogenetic composition and physiological activity of bacterioplankton communities in two different rivers in Southeast Asian countries, the Kelang River basin in Malaysia and Chao Phraya River in Thailand, which are polluted by untreated or incompletely treated sewage, were analyzed by fluorescent in situ hybridization (FISH) and FISH combined with the direct viable count technique (DVC-FISH). The results were also compared with those from temperate zone habitats in eutrophic rivers in Osaka, Japan. FISH detected 56% to 78% of total cells with the probe EUB338 targeted for the domain *Bacteria* in samples from the Kelang River basin, compared with 14% to 33% in samples from the Chao Phraya River. DVC-FISH with an antibiotic cocktail increased the fraction of bacteria detectable with EUB338 in the Chao Phraya River (72% to 75% of total bacteria), while no appreciable change was found in samples from the Kelang River basin. These results show that in situ physiologic activity of resident bacteria was generally high in the Kelang River basin and low but present in the Chao Phraya River. Bacterial community structures in both rivers were dominated by the beta (5% to 39%) and gamma (4% to 41%) subclasses of *Proteobacteria*. In river water samples from Osaka, bacterial community structures determined by FISH were dominated by the beta subclass, but those determined by DVC-FISH were dominated by both beta (26% to 39%) and gamma (17% to 47%) subclasses. This result implies that in situ physiological activity of the gamma subclass is low in the eutrophic river in Osaka, but those bacteria have the potential for cell division.

Key words —— river, bacterial community composition, physiological activity, fecal bacteria

INTRODUCTION

Tropical rivers in Southeast Asia have been seriously polluted by recent industrialization and urbanization.[1–5] Rivers are the major source of fresh water available for many purposes, *e.g.*, consumption, industry, and agriculture. There is an increasing awareness of the necessity for conserving fresh water resources. Microbial communities play important roles in the biodegradation of pollutants derived from human activities, and contribute to natural self-purification. An understanding of the structure and dynamics of microbial communities in tropical rivers is thus a necessity. Microbial ecology in tropical zones is being explored, but current knowledge is limited, especially in tropical nations in Southeast Asia which are rapidly achieving economic growth.[6–8]

The determination of bacterial community composition and their activities in nature is fundamental but has long been a challenge to microbial ecologists. Fluorescent in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes has recently provided information about absolute abundance, morphology, and cell size of bacteria with defined phylogenetic affiliations,[9,10] and been applied to the investigation of community composition in lakes, oceans, activated sludge, and drinking water.[11–14] Recent studies have demonstrated that microbial communities in various aquatic systems are dominated by bacteria that are phylogenetically affiliated with the alpha and beta subclasses of the *Proteobacteria* and with members of the *Cytophaga-Flavobacterium* cluster.[15–17]

The signal conferred by fluorescently labeled rRNA-targeted probes to fixed cells is highly dependent on cellular rRNA content, which is an indicator of physiologic activity.[18,19] The application of

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FISH to oligotrophic environments remains limited because the cellular abundance of rRNA in resident bacteria is much lower than in cultured ones.[10] Consequently, detection yields relative to a DNA-specific fluorochrome, *e.g.*, 4′,6-diamidino-2-phenylindole (DAPI) with a domain-specific probe, *e.g.*, EUB338, in oligotrophic samples have not been sufficiently high to demonstrate the community structure of bacterial populations.[15,20] Another limitation of FISH is that it does not provide much information on the viability of target cells. A direct correlation between viability and cellular rRNA content was observed in *Escherichia coli* cells undergoing starvation,[21] but was not seen in starved *Azotobacter agilis* cells,[22] and the breakdown of ribosomes and rRNA is not necessarily related to loss of viability.[23] Therefore the identification of bacterial cells by FISH may not correspond to viability in the habitat.

Simultaneously determining in situ the identities and viability of individual bacterial cells within complex microbial communities will be of great assistance in understanding which cells are responsible for bacterial activities, *e.g.*, biodegradation of xenobiotics. One approach to achieve this purpose is the combination of FISH with the direct viable count technique (DVC-FISH).[12,24] The original DVC method is based on the incubation of samples with a single antibiotic (nalidixic acid) and nutrients.[25] Recently a cocktail of several antibiotics has been employed to inhibit cell division of most bacterial species in the DVC procedure.[26] DVC-FISH with several antibiotics has great potential to reduce the negative aspects described above and provide an in situ hybridization strategy.

In this study, DVC-FISH was applied to improve the sensitivity of the standard FISH and evaluate the viability of a phylogenetically defined population in urban rivers in Southeast Asia. Modified DVC-FISH with domain- and group-specific oligonucleotide probes was used to investigate the community composition and physiologic activity of bacterioplankton in two different rivers that are polluted by untreated or incompletely treated sewage, the Kelang River basin which is shallow and narrow (Malaysia), and the Chao Phraya River, which is deep and wide (Thailand). The results were also compared with those from eutrophic rivers in Osaka, Japan.

MATERIALS AND METHODS

Sampling Stations —— Water samples were collected at five sites (KL1, KL3, KL4, SA6, K7) in the Kelang River basin, Malaysia (Fig. 1A). KL1 (3°10′17″N, 101°41′54″E), KL3 (3°10′17″N, 101°41′63″E), and KL4 (3°8′85″N, 101°41′70″E) are located in residential areas (KL1, KL3) and a commercial area (KL4) in the capital of Kuala Lumpur. SA6 (3°2′30″N, 101°32′9″E) is located in an industrial area in Shah Alam. K7 (3°2′75″N, 101°26′78″E) is located in a commercial area in Kelang. Domestic water flows directly into this river. Chlorine treatment of sewage is not carried out at sewage plants in Malaysia before it flows into rivers. The water quality of the Kelang River basin has been monitored by ASMA Co. (Malaysia), and biochemical oxygen demand (BOD) was 6 to 17 mg/l (average, 10 mg/l) in Kuala Lumpur and 2 to 15 mg/l (average, 7.1 mg/l) in Kelang in 1999. In the Chao Phraya River, water samples were collected at three sites, CP1 (13°26′50″N, 100°22′32″E), CP2 (13°44′36″N, 100°29′85″E), and CP3 (13°46′92″N, 100°30′11″E), which are located in residential areas in the capital of Bangkok (Fig. 1B). Some sewage plants have been constructed in Bangkok, but the total number is not yet sufficient, and drainpipes are sometimes incomplete. The water quality of the Chao Phraya River has been monitored by the Pollution Control Department of Thailand, and BOD was 1.1 to 8 mg/l (average, 3.5 mg/l) in metropolitan Bangkok in 1999. The Kelang River basin and Chao Phraya River are polluted by untreated or incompletely treated sewage.

Sampling sites in Osaka, KN (34°44′91″N, 135°29′81″E), NY (34°41′75″N, 135°31′90″E), HR (34°38′57″N, 135°32′34″E), and YM (34°35′92″N, 135°32′58″E), are located in an industrial (KN) and commercial areas (NY, HR, YM) (Fig. 1C). Sewage plants have been constructed in Osaka, but sampling sites are polluted by organic carbon from treated sewage. BOD is 10 to 12 mg/l in these sites. Water samples were collected from the surface at each site and the physicochemical and microbiologic parameters were determined from December 1998 to October 1999. Representative physicochemical characteristics of the river water are described in Table 1.

Plate Counting —— The number of colony-forming units was determined by spreading dilute river water samples on R2A medium,[27] which were incubated at 30°C (samples from Malaysia and Thailand) or 25°C (samples from Japan) for 1 week before

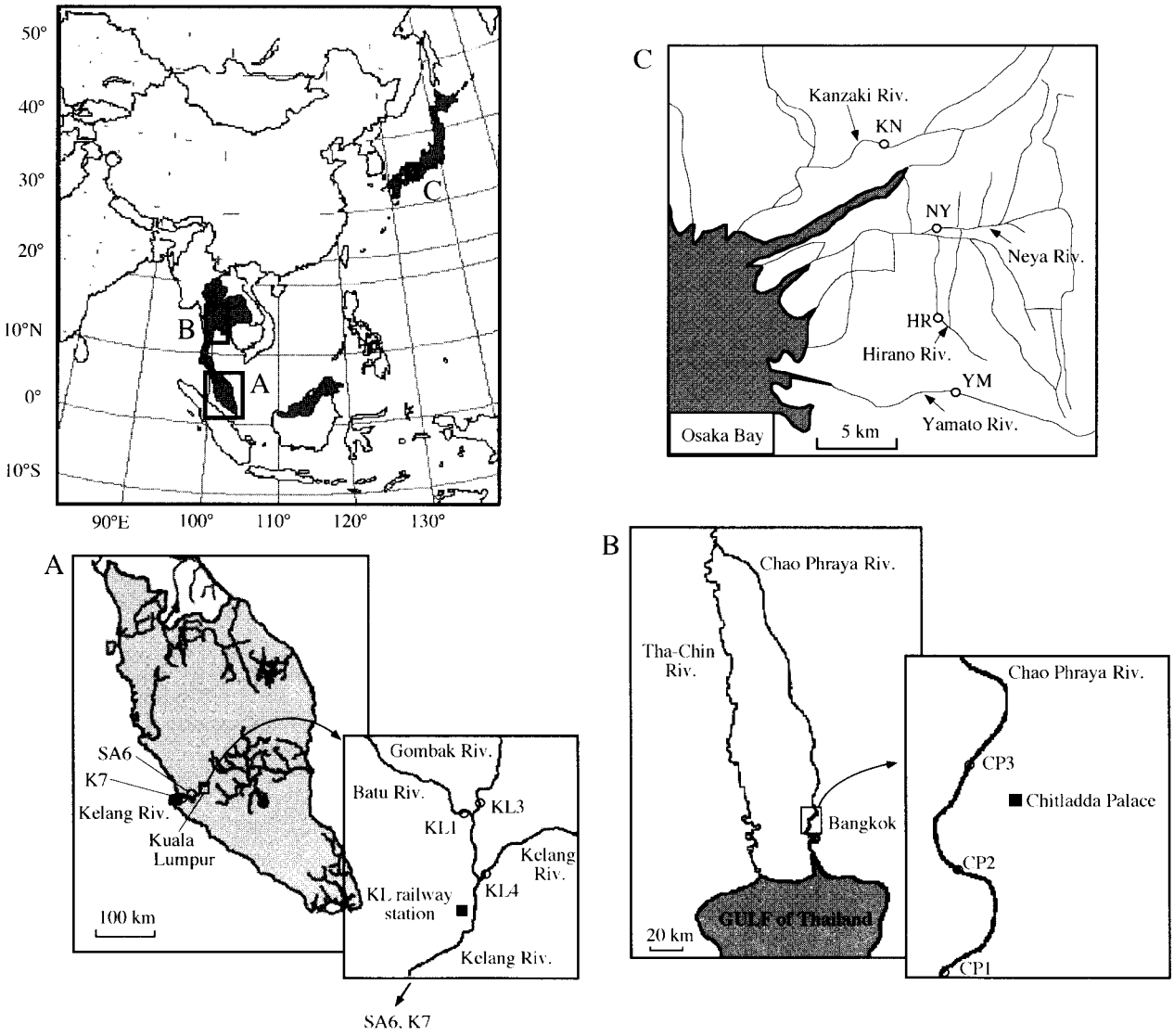

Fig. 1. Map of Sampling Sites in the Kelang River Basin (A), Chao Phraya River (B), and Rivers in Osaka (C)

counting.

Oligonucleotide Probes —— The probe sequences, hybridization conditions, and references are given in Table 2. Unlabeled probes BET42a and GAM42a were used with competitor oligonucleotides. ES445 targeted for *Escherichia* and *Shigella* was designed in this study because these genera are phylogenetically related and difficult to distinguish with 16S rRNA sequences. The design of probe ES445 was carried out as described previously.[31] In the GenBank database (Release 115.0) and Ribosomal RNA Database Project,[32] sequences of two nontarget strains, *Hafnia alvei* and *Serratia marcescens*, showed a complete match with probe ES445, but these strains ordinarily originate from a fecal source. Our hybridization conditions could distinguish target strains (*E. coli* K-12 W3110 and *E. coli* O157 : H7 ATCC43888) from nontarget strains (two mismatches: *Enterobacter gergoviae* GIFU 10615, *Klebsiella oxytoca* GIFU 3162; more than three mismatches: *Acinetobacter calcoaceticus* ATCC 23055, *Acinetobacter haemolyticus* ATCC 17906, *Acinetobacter johnsonii* ATCC 17909, *Acinetobacter junii* ATCC 17908, *Acinetobacter lwoffii* ATCC 15309, *Aeromonas caviae* GIFU12084, *Burkholderia cepacia* RIMD1644001, *Flavobacterium johnsoniae* ATCC 17061, *Pseudomonas putida* RIMD1626003, *Pseudomonas mendocina* GIFU1953, *Sphingobacterium thalpophilum* IFO14963, *Vibrio vulnificus* ATCC 27562).

FISH —— Twelve percent (w/v) paraformaldehyde in phosphate buffer saline (PBS) was added to the

Table 1. Physicochemical Characteristics of River Water Samples

Sampling site	Date	A.T.[a] (°C)	W.T.[b] (°C)	pH	EC[c] (μS/cm)	TOC[d] (mg/l)
Kuala Lumpur, Malaysia						
KL1	14 Oct. 1999	25.0	27.1	7.5	200	5.4
KL3	14 Oct. 1999	25.1	25.6	7.4	189	4.1
KL4	14 Oct. 1999	24.6	25.7	7.6	195	4.2
SA6	14 Oct. 1999	31.8	27.5	7.3	219	6.5
K7	14 Oct. 1999	29.0	26.7	7.3	159	5.5
Bangkok, Thailand						
CP3	12 Oct. 1999	30.1	30.8	7.8	220	4.6
CP2	12 Oct. 1999	29.5	30.7	7.9	200	4.7
CP1	12 Oct. 1999	29.4	30.6	7.7	230	5.5
Osaka, Japan						
HR	27 Sept. 1999	29.3	26.4	7.6	700	9.8
YM	27 Sept. 1999	27.1	27.4	7.8	420	5.6
KN	27 Sept. 1999	23.8	26.3	7.8	387	6.3
NY	27 Sept. 1999	27.4	27.5	7.6	394	5.1

a) A.T., ambient temperature. *b*) W.T., water temperature. *c*) EC, electrical conductivity. *d*) TOC, total organic carbon.

Table 2. Oligonucleotide Probes Used in This Study

Probe	Specificity	Sequence (5′-3′)	Tartget position[a]	FA[b]	NaCl[c]	Reference
EUB338	*Bacteria*	GCTGCCTCCCGTAGGAGT	16S, 338–355	0%	0.9 M	9
NON	Negative control	ACTCCTACGGGAGGCAGC	16S, 338–355	0%	0.9 M	19
ALF1b	Alpha subclass of Proteobacteria	CGTTCG(C/T)TCTGAGCCAG	16S, 19–35	20%	0.225 M	28
BET42a	Beta subclass of Proteobacteria	GCCTTCCCACTTCGTTT	23S, 1027–1043	35%	80 mM	28
GAM42a	Gamma subclass of Proteobacteria	GCCTTCCCACATCGTTT	23S, 1027–1043	35%	80 mM	28
CF319	*Cytophaga-Flavobacterium* cluster of CFB-phylum	TGGTCCGT(G/A)TCTCAGTAC	16S, 319–336	15%	80 mM	29
BAC303	*Bacteroides* cluster of CFB-phylum	CCAATGTGGGGGACCTT	16S, 303–319	0%	0.9 M	29
ES445	*Escherichia-Shigella* and relatives	CTTTACTCCCTTCCTCCC	16S, 445–462	45%	80 mM	Present study
ARCH915	*Archaea*	GTGCTCCCCCGCCAATTCCT	16S, 915–935	25%	0.225 M	30

a) *E.coli* numbering. *b*) Percentage of formamide (FA) in hybridization buffer. *c*) Concentration of NaCl in washing buffer.

collected samples in a final concentration of 3% (w/v), and cells were fixed at 4°C overnight. Cells were concentrated from water samples (0.5 to 1.5 ml) on polycarbonate filters (diameter, 25 mm; pore size, 0.2 μm; ADVANTEC, Japan.). The filters were subsequently washed with 3 ml each of sterilized water, which was removed by applying a vacuum. Air-dried filters are ready for hybridization and can be stored at –20°C. Each filter was cut into 8 sections. Then each filter section was placed on glass slides and covered with 50 μl of hybridization solution containing NaCl 0.9 M, Tris–HCl 20 mM (pH 7.5), EDTA 5 mM, 0.01% (w/v) sodium dodecyl sulfate, a variable concentration of formamide, and 50 ng of CY3-labeled oligonucleotide. The filter sections were incubated at 46°C for 3 h in an equilibrated chamber for hybridization, and then they were transferred to a Eppendolf tube containing 300 μl of prewarmed (48°C) washing solution (a variable concentration of NaCl, Tris–HCl 20 mM [pH 7.5], EDTA 5 mM, 0.01% [w/v] sodium dodecyl sulfate) for incubation without shaking at 48°C for 30 min. The filter sections were dried on paper and transferred to a Eppendorf tube containing DAPI 300 μl solution (1 μg/ml in sterilized water, Sigma) or SYBR Green II solution (1×10^{-4} stock solution, Molecular Probes Inc.) for 5 min at room temperature in the dark. Subsequently, they were gently washed in 10 ml of sterilized water, dried on paper, and mounted on glass slides with immersion oil (Olympus, Japan). Bacterial cells on the filter sections were observed with an BX 50 (Olympus, Japan) equipped with a 100 W mercury and filter sets (DAPI [U-MWU]; SYBR Green II [U-MNIBA]; CY3 [U-

MWIG]). Cell enumeration and data processing were performed as described by Glöckner *et al.*[17] Probe-specific cell counts are presented as the percentage of cells visualized by DAPI or SYBR Green II, and the mean abundances and standard deviations were calculated. All counts were corrected by subtracting the counts obtained with the negative control probe NON.

DVC-FISH —— River water samples were incubated in yeast extract solution (50 μg/ml) containing nalidixic acid (20 μg/ml), piromidic acid (10 μg/ml), pipemidic acid (10 μg/ml), cephalexin (10 μg/ml), and ciprofloxacin (0.5 μg/ml).[26] Incubation was performed statically in the dark at 30°C (samples from Malaysia and Thailand) or 25°C (samples from Japan), and samples were fixed with paraformaldehyde (final concentration: 3%) after 18 h incubation for DVC. Then in situ hybridization of incubated samples was performed as described above.

RESULTS AND DISCUSSION

Total Cell Counts and Plate Counts

SYBR Green II was used for nucleic acid staining of samples from tropical habitats in this study. The fluorochrome most often used for total direct counting and counterstaining in FISH is DAPI.[33] Although DAPI was applied to tropical river samples, the amount of suspended solid in the samples caused a high background with epifluorescent microscopy.

Total cell counts and the percentages of colony-forming bacteria to total bacteria in the river water samples are shown in Fig. 2. The total cell counts found in the Kelang River basin (3×10^6 to 1×10^7 cells/ml), the Chao Phraya River (2×10^6 to 4×10^6 cells/ml), and in rivers in Osaka (4×10^6 to 1×10^7 cells/ml) were within the range reported for eutrophic river systems.[34] Culturable bacteria accounted for 5% to 30% of the total bacteria in river water samples taken in Osaka and 5% to 8% in the Chao Phraya River, while estimates of culturable bacteria in the Kelang River basin were extremely high, constituting 19% to 58% of total bacteria. For aquatic habitats, it has been reported that direct microscopic counts exceed viable plate counts by several orders of magnitude.[25,35] This has also been found in sediment and soil samples.[35,36] Plate counting on R2A media yielded a higher number of colony-forming bacteria,[27] especially in eutrophic river samples.[34] Our results imply that the physiologic state of resident bacteria in the Kelang River basin differs greatly from that in other aquatic habitats.

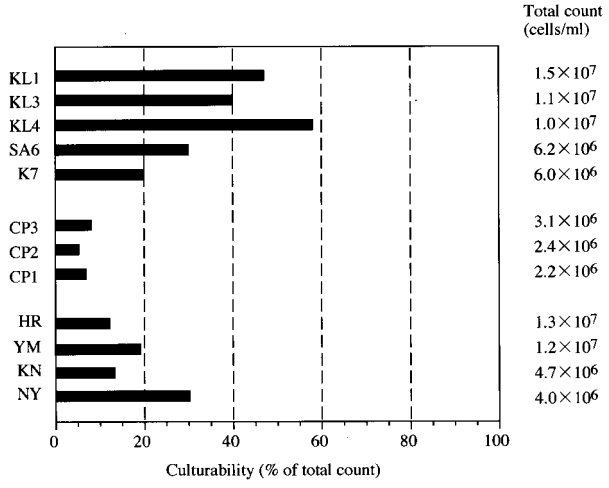

Fig. 2. Culturable Bacteria in River Water Samples Measured as Colony-Forming Units on R2A Medium

FISH with Domain-Specific Probes

The community composition of the different bacterial groups as a fraction of total counts was determined by FISH (Fig. 3A) and DVC-FISH (Fig. 3B). The label "Not identified" refers to the share of bacteria detectable by the probe EUB338 that could not be explained by the sum of bacteria detectable with the four group-specific probes (ALF1b, BET42a, GAM42a, and CF319). In the Kelang River basin between 56 ± 22% and 78 ± 18% of total cells could be visualized by FISH with the probe EUB338 targeted for the domain *Bacteria*, and between 44 ± 4% and 61 ± 9% of DAPI-stained cells in rivers in Osaka were detected by EUB338 (Fig. 3A). Detection frequencies were lowest in the Chao Phraya River (14 ± 5% to 33 ± 13%). *Archaea* represented 1 ± 1% to 3 ± 1% in river water samples from Osaka, and were also detected in samples from site KL4 (1 ± 0.9%). This supports other findings on the widespread occurrence but low abundance of *Archaea* in aquatic systems.[16,17]

DVC-FISH with the antibiotic cocktail increased the fraction of bacteria detectable with EUB338 in the Chao Phraya River (72 ± 11% to 75 ± 11% of total bacteria) and in rivers in Osaka (75 ± 11% to 77 ± 13% of total bacteria), while changes in detection frequencies were not appreciable in samples from the Kelang River basin. Total cell count did not change during incubation for DVC in any samples (data not shown). The increasing fraction of bacteria detectable by EUB338 after incubation

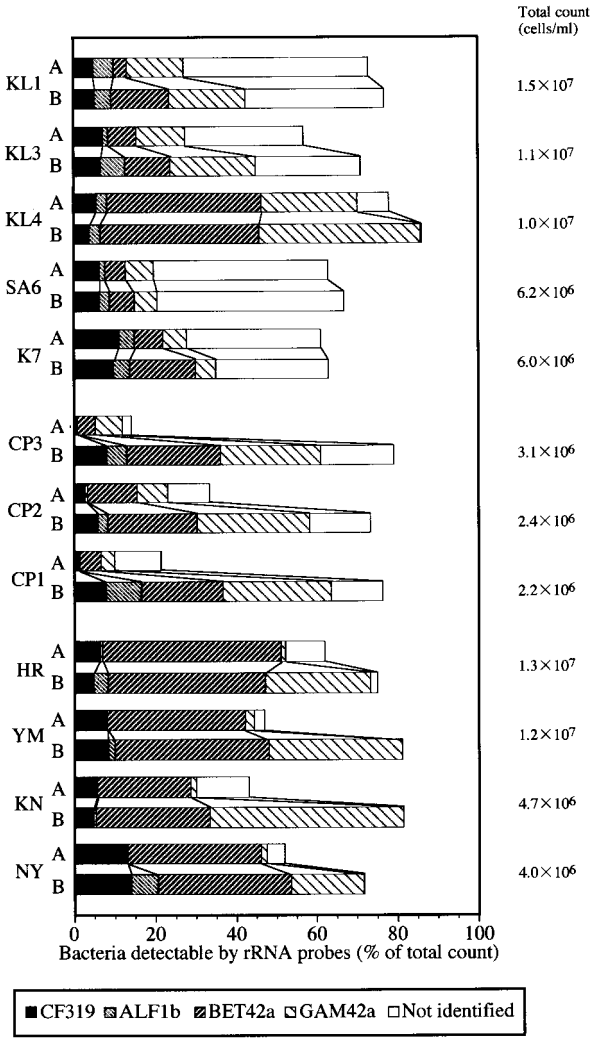

Fig. 3. Bacterial Community Composition in River Water Samples Determined by FISH (A) and DVC-FISH (B)

for DVC identifies the existence of cells metabolically activated by the addition of nutrients, because incubation for DVC encouraged an increase in cellular rRNA content and the signals conferred by fluorescent-labeled rRNA-targeted probes increased above the detection limit.[12,24] The higher detection frequencies of DVC-FISH demonstrated that in situ physiologic activity of certain bacterial populations with increased rRNA content during incubation for DVC was low, but most bacteria were viable and survived in the Chao Phraya River and rivers in Osaka. The results from the samples in the Kelang River basin indicate that in situ physiologic activity of resident bacteria in these rivers was generally higher than in other rivers; thus a change in the detection frequencies might not be appreciable before and after incubation for DVC.

Although no significant differences in characteristics such as electric conductivity, total organic carbon, and total cell counts were found among samples from the Kelang River basin, the Chao Phraya River, and rivers in Osaka, indicators of physiologic activity determined by plate counting and hybridization efficiency showed that the physiological activity of resident bacteria in the Kelang River basin was generally high. In the Kelang River basin, a large amount of untreated sewage flows into shallow and narrow rivers. Moreover, chlorination treatment is not carried out at sewage plants in Malaysia. For this reason, enriched cells in untreated or incompletely treated sewage might contaminate the river and in situ physiologic activity of resident bacteria might be generally high in the Kelang River basin.

FISH with Group-Specific Probes

Bacteria in river water samples from Osaka frequently hybridized with probe BET42a (25 ± 4% to 43 ± 9% of total cells) and did not hybridize with probe GAM42a (less than 1.8% of total cells), whereas bacterial community structures in the Kelang River basin and Chao Phraya River were generally dominated by members of both beta (2.3 ± 3% to 37 ± 8%) and gamma (3.2 ± 1% to 27 ± 15%) subclasses of *Proteobacteria* (Fig. 3A). In most studies with rRNA-targeted in situ hybridization of samples from temperate habitats, it has been demonstrated that the beta subclass of *Proteobacteria* constitute a dominant fraction in freshwater systems. Members of the *Cytophaga-Flavobacterium* cluster were the most abundant group detected in the marine systems, and they were also important in freshwater systems. Members of the gamma subclass were reported to constitute only a small fraction in various water systems.[13,15–17] This phenomenon was also observed in temperate-habitat samples from Osaka, but it was not found in tropical-habitat samples from the Kelang River basin and Chao Phraya River. Our results indicate the significance of the gamma subclass bacteria in these rivers.

The results with DVC-FISH showed that the gamma subclass was abundant in water samples from Osaka (Fig. 3B). DVC-FISH revealed that the gamma subclass constituted a considerable cell fraction with low cellular rRNA content in eutrophic rivers in Osaka. Thus members of this subclass might survive in various water systems in temperate zones despite the fact that estimates of the gamma subclass determined by standard FISH were significantly lower than those for other members.[15,17] The

positive selection for gamma subclass bacteria on agar plates is a well-known phenomenon that has been analyzed by FISH.[11] Many members of this group adapt to high nutrient concentrations, and therefore grow well under laboratory conditions.[37] Our results also show that they respond well to addition of nutrients for DVC incubation. It should be noted as well that members of the gamma subclass with low physiologic activity were underestimated by the standard FISH technique, and modified DVC-FISH with the antibiotic cocktail may be useful for the detection of viable bacteria with low physiologic activity in aquatic environments.

Abundance of Fecal Bacteria

The abundance of bacteria expected to be from a fecal source was investigated to clarify the differences in community composition in these rivers. Coliforms have been used as the standard indicator of recent fecal contamination under most conditions in temperate fresh water. In tropical nations, they have been used to monitor contamination levels despite reports that free-living coliforms may be indigenous to some tropical waters and can't be distinguished from those from a fecal source.[6,8] In human intestinal microflora, the anaerobe *Bacteroides* is the most common genus and it outnumbers the coliforms.[38] Conventional coliform tests as well as in situ identification with rRNA-targeted probes for *E. coli* and the *Bacteroides* group were used in this study. Estimates of coliforms in samples from the Kelang River basin were much higher than in those from other sites, and detection with BAC303 probes revealed that the *Bacteroides* group accounted for $1.3 \pm 0.5\%$ to $6.9 \pm 2\%$ of the total bacteria (Table 3). Members of *Bacteroides* are anaerobes and can't survive in river water.[39] The range of dissolved oxygen at the sites in the Kelang River basin was 0.5 to 6 mg/ml. In addition, the abundance of coliforms and *Bacteroides* at upstream sites in the Kelang River basin was lower than at our sampling sites in Kuala Lumpur by one order of magnitude. It is highly probable that the waters of the Kelang River basin are grossly contaminated by fecal flora, which might then constitute a considerable cell fraction.

In samples from the Chao Phraya River, estimates of the *Escherichia-Shigella* group determined with probe ES445 were higher than those of the *Bacteroides* group, and accounted for $1.0 \pm 1\%$ to $8.9 \pm 3\%$ of total cells. It is difficult to conclude that the presence of cells detectable by probe ES445 corresponds to the source of fecal pollution in tropical rivers, but the high abundance of anaerobes of the *Bacteroides* group indicates fecal pollution in the Chao Phraya River because the range of dissolved oxygen at these sites was 1 to 4 mg/ml. These results with rRNA-targeted in situ hybridization suggest that members of the *Escherichia-Shigella* group from a fecal source may survive and constitute a considerable cell fraction in the Chao Phraya River. On the other hand, the abundance of coliforms and the *Bacteroides* group in the Chao Phraya River was significantly lower than in the Kelang River basin. This may be because waste water is diluted in the deep and wide Chao Phraya River more effectively

Table 3. Abundance of Fecal Bacteria in River Water Samples

Site	Coliform		BAC303[b]		ES445[c]	
	10^3/ml (%)[a]		10^3/ml (%)		10^3/ml (%)	
KL1	> 24	(> 0.5)	64	(1.3 ± 0.5)	—[d]	(< 0.1)
KL3	5.4	(0.15)	99	(2.7 ± 3)	—	(< 0.1)
KL4	> 24	(> 0.30)	500	(6.9 ± 2)	14	(0.2 ± 0.6)
SA6	2.4	(0.06)	120	(3.2 ± 3)	87	(2.3 ± 2)
K7	9.2	(0.30)	65	(1.8 ± 2)	22	(0.6 ± 1)
CP3	0.54	(0.017)	12	(0.4 ± 0.6)	280	(8.9 ± 3)
CP2	0.35	(0.015)	17	(0.7 ± 0.7)	24	(1.0 ± 1)
CP1	0.35	(0.016)	18	(0.8 ± 0.8)	150	(6.9 ± 2)
HR	0.35	(0.0027)	—	(< 0.1)	—	(< 0.1)
YM	0.92	(0.0080)	—	(< 0.1)	—	(< 0.1)
KN	0.35	(0.0075)	—	(< 0.1)	—	(< 0.1)
NY	0.35	(0.0088)	—	(< 0.1)	—	(< 0.1)

a) % of total count. Mean and standard deviation were calculated from the counts on each filter section. *b*) *Bacteroides* groups detemined by DVC-FISH. *c*) *Escherichia-Shigella* and relatives detemined by DVC-FISH. *d*) Not detected.

than in the shallow and narrow Kelang River. The depths and widths of the Chao Phraya River at our sampling sites are 10 to 18 m and approximately 200 m, respectively. Those of the Kelang River are approximately 2 m and less than 20 m, respectively, at sampling sites in Kuala Lumpur, and approximately 2 m and 100 m in other sites.

In the samples from Osaka, estimates of the *Escherichia-Shigella* group and *Bacteroides* group were below detection limits. This may be due to the effect of sewage treatment.

The abundance and activity of fecal bacteria in urban rivers in Malaysia and Thailand indicate that understanding the dynamics of these microbes in the environment is important to improve the bacterial water quality of these rivers and avoid threats to public health from contaminated water.

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