# Distribution of fecal bacterial groups in the river and lake water in the city of Hanoi, Vietnam

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## Abstract

Fluorescent in situ hybridization (FISH) was used to study the distribution of *Escherichia-Shigella* clusters and *Bacteroides* groups as an indicator of fecal contamination in the Red River, lakes and canals of Hanoi, thus assessing city water sources for pollution with fecal bacteria. The number of active bacteria was estimated by FISH. The percentage of bacteria detected with an EUB338 probe compared to the total counts was over 50% in all samples. In canal water samples, approximately 75% of DAPI-stained cells were detected with this probe. The number of *Bacteroides* group bacteria was also determined by FISH. At eight sampling stations, *Bacteroides* groupswere detected. The numbers were high in canal samples. On the other hand, the abundance of the *Bacteroides* group. in the Red River was significantly lower than the canals. In Vietnam, monitoring fecal bacterial groups, especially *Bacteroides* group. with FISH method is useful to improve the bacterial water quality and avoid threats to

public health from contaminated water.

## Introduction

Water contamination is an important public health issue. Assessment and control of bacterial quality is necessary in order to reduce waterborne infectious illness. Fecal bacteria, in particular, are important pathogens widely recognized as major indicators of the hygienic quality of water. Tropical river waters in Southeast Asia have been polluted by recent urbanization and industrialization. The water sources have been contaminated with fecal bacteria[3]. The city of Hanoi in Vietnam also has been urbanized in recent decades, however, these bacterial contamination in its water sources has been poorly understood. In this study, fluorescent in situ hybridization (FISH) was used to study the distribution of *Escherichia-Shigella* clusters and *Bacteroides* groups as an indicator of fecal contamination in the Red River, lakes and canals of Hanoi, thus assessing city water sources for pollution with fecal bacteria

## Materials and Methods

### Water samples

Water samples were collected from the river, lakes and canals in Hanoi city from 28 to 30 November 2000. All sampling sites were shown in Fig. 1. One liter of water was collected just below the surface.

### Most probable number (MPN) counts of coliforms

Number of viable coliforms were estimated with MPN dilutions in liquid medium.

The lactose broth (3 g meat extract, 10 g peptone, 0.024 g bromothymol blue, 5 g lactose in 1 L [pH7.0-7.4]) was used. The medium was dispensed into the test tube with the Durham tube. Ten-fold serial diluted samples were inoculated into the lactose broth and incubated for 24 h at 37C. The positive tube in which coliform was present was judged by the gas production in the Durham tube.

Cell fixation

All samples were fixed with 4 % of paraformaldehyde in phosphate-buffered saline (PBS) at 4C for 16 h. Fixed cells were trapped onto 0.2 um-pore polycarbonate filters (Toyo Roshi Kaisha, Ltd, Tokyo, Japan) and stored at –20C.

Oligonucleotide probes and fluorescent in situ hybridization

The probe sequences, hybridization conditions and references are given in Table 1. The filter with fixed bacterial cells was placed on glass slide and covered with 50 ul of hybridization solution containing 0.9 M NaCl, 20 mM Tris-HCl[pH7.5], 5 mM EDTA, 0.01%sodium dodecyl sulfate (SDS), variable concentration of formamide and 50 ng of CY3-labeled oligonucleotide. The filters were incubated at 46C for 3 h in an equilibrated chamber for hybridization, and then they were transferred to a plastic sampling tube (1.5 ml volume) containing 300 ul of prewarmed (48C) washing solution Containing 20 mM Tris-HCl[pH7.5], 5 mM EDTA, 0.01%SDS and variable concentration of NaCl for incubation without shaking at 48C for 30 min. The filters were dried on paper and transferred to a fresh tube containing 300 ul of DAPI solution (1 ug/ml) After 5 min incubation in the dark, Bacterial cells on the filters were observed with BX50 (Olympus , Japan) with a 100 W mercury and filter sets.

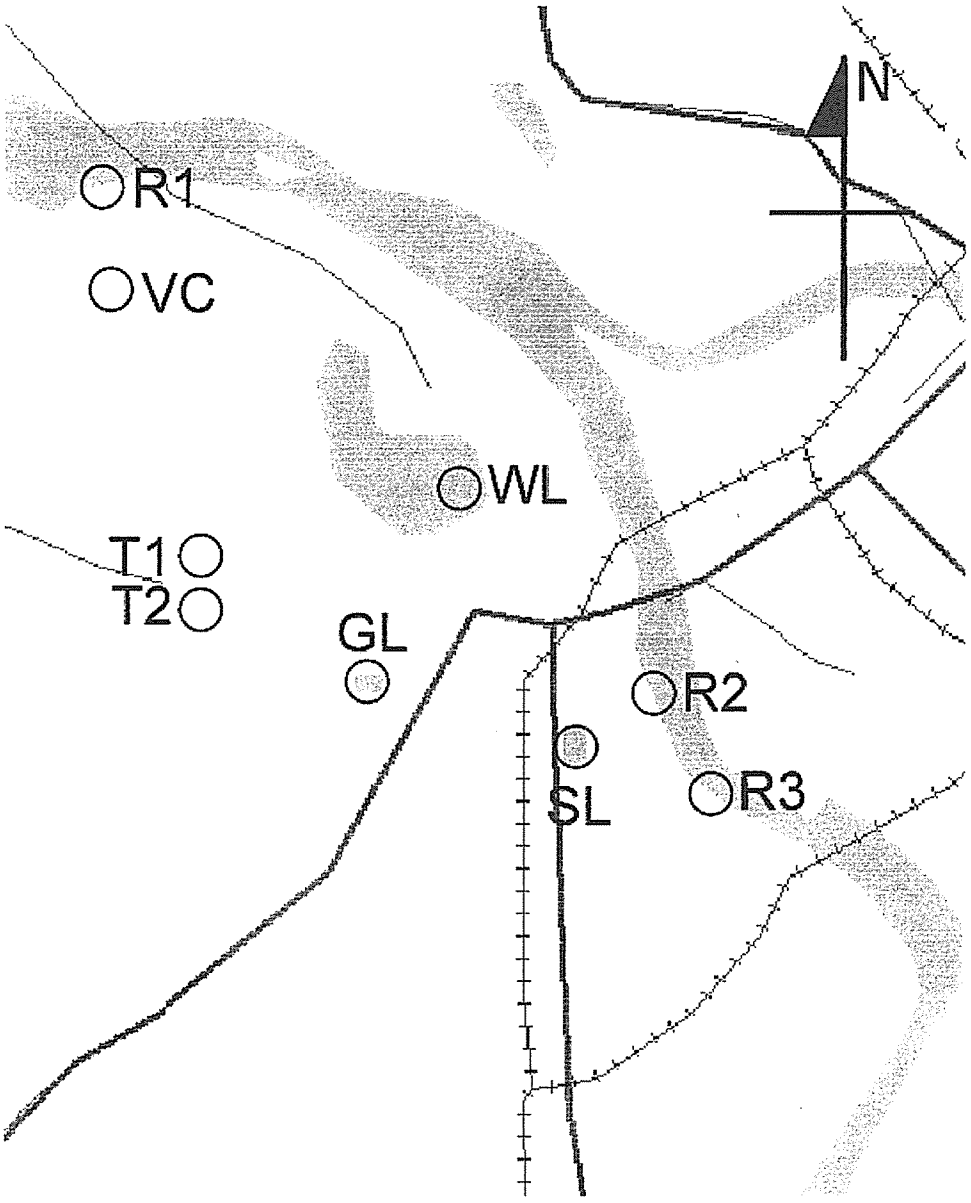


Fig. 1 Sampling stations in Hanoi

## Results and discussion

The total cell counts found in canal and lake water samples ranged from $9.0 \times 10^6$ to $2 \times 10^7$. These values were similar to those reported for river systems in Thailand and Malaysia[2,3]. In contrast, levels found in Red River samples were considerably lower. The number of active bacteria was estimated by FISH. The percentage of bacteria detected with an EUB338 probe compared to the total counts was over 50% in all samples. In canal water samples, approximately 75% of DAPI-stained cells were detected with this probe. These results imply that most bacteria in the samples collected in Hanoi were physiologically active and contained many copies of ribosomal RNA for protein synthesis.

The distribution of fecal bacterial groups in the water samples was investigated. Coliform numbers in all of the Red River (R1, R2, R3) and lake samples (WL, SL, GL) were less than 500 MPN / ml. These values are less than those reported in the urban rivers of Japan and Thailand. However, coliform numbers in all canal samples were higher than any obtained from river and lake samples. The ratio of coliform number to *Escherichia-Shigella* probe counts was less than 10% for all samples. This indicates that the majority of *E. coli* were quite active but could not be found in the media. 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 cannot be distinguished from those of contamination. Thus, the number of *Bacteroides* group bacteria, a common genus in human intestinal microflora, was also determined by FISH. At eight sampling stations,

*Bacteroides* groupswere detected. The numbers were high in canal samples (VC, T1 and T2). These sampling stations were surrounded by houses and sewage line was not equipped well, so that domestic wasted water flowed in these canals. In some sites of Vietnam, canalization was not fully equipped thus the wastewater of the private

Table 1 Probes used in this study

| Probe | Specificity | Sequence (5'-3') | Target position[a] | FA[b] | NaCl[c] | Referrence |
|---|---|---|---|---|---|---|
| EUB338 | *Bacteria* | GCTGCCTCCCGTAGGAGT | 16S 338-355 | 0% | 900 mM | 1 |
| BAC303 | *Bacteroides* cluster of CFB-phylum | CCAATGTGGGGGACCTT | 16S 303-319 | 0% | 900 mM | 4 |
| ES445 | *Escherichia-Shigella* and relatives | CTTTACTCCCTTCCTCCC | 16S 445-462 | 45% | 80 mM | 3 |

Table 2 Abundance of fecal bacteria

| Sampling station | Sampling data | Total counts (cells/ml) | Coliform (MPN/ml) | EUB[a] (cells/ml) | ES[b] (cells/ml) | BAC[c] (cells/ml) |
|---|---|---|---|---|---|---|
| R1 | Nov 30, 2000 | $4.3 \times 10^5$ | $2.4 \times 10^0$ | $2.2 \pm 0.4 \times 10^5$ | $1.7 \pm 1.5 \times 10^3$ | $1.8 \pm 0.1 \times 10^3$ |
| R2 | Nov 28, 2000 | $6.3 \times 10^5$ | $4.4 \times 10^{-1}$ | $4.6 \pm 0.4 \times 10^5$ | $3.1 \pm 1.4 \times 10^3$ | N.D. |
| R3 | Nov 29, 2000 | $6.2 \times 10^5$ | $4.6 \times 10^1$ | $3.7 \pm 0.3 \times 10^5$ | $4.6 \pm 3.0 \times 10^2$ | $2.4 \pm 1.4 \times 10^3$ |
| WL | Nov 28, 2000 | $1.8 \times 10^7$ | $7.3 \times 10^1$ | $1.3 \pm 0.1 \times 10^7$ | $8.9 \pm 3.9 \times 10^4$ | $3.7 \pm 1.2 \times 10^5$ |
| SL | Nov 29, 2000 | $9.0 \times 10^6$ | $9.3 \times 10^1$ | $6.4 \pm 0.8 \times 10^6$ | $5.3 \pm 1.9 \times 10^4$ | $4.9 \pm 2.4 \times 10^4$ |
| GL | Nov 30, 2000 | $1.7 \times 10^7$ | $4.6 \times 10^2$ | $9.0 \pm 0.2 \times 10^6$ | $1.2 \pm 0.2 \times 10^5$ | $9.5 \pm 4.9 \times 10^4$ |
| T1 | Nov 29, 2000. | $1.7 \times 10^7$ | $2.4 \times 10^3$ | $1.3 \pm 0.3 \times 10^7$ | $6.3 \pm 3.4 \times 10^4$ | $1.3 \pm 0.0 \times 10^6$ |
| T2 | Nov 28, 2000 | $1.1 \times 10^7$ | $9.3 \times 10^2$ | $7.8 \pm 0.7 \times 10^6$ | $9.3 \pm 6.9 \times 10^4$ | $2.7 \pm 0.4 \times 10^5$ |
| VC | Nov 30, 2000 | $2.0 \times 10^7$ | $1.1 \times 10^4$ | $1.4 \pm 0.1 \times 10^7$ | $7.7 \pm 2.1 \times 10^5$ | $9.6 \pm 8.2 \times 10^5$ |

a) Bacteria detected by rRNA-targeted FISH with EUB probe, b) Bacteria detected by rRNA-targeted FISH with ES probe, c) Bacteria detected by rRNA-targeted FISH with BAC302 probe, N.D.: not detected

T houses flows directly into the canal At T1 and T2, dissolved oxygen was very exiguous, therefore the anaerobic environments were suitable for survival of Bacteroides group.. These results, in conjunction with ES count, suggests that these canals were highly polluted with fecal contamination. On the other hand, the abundance of the *Bacteroides* group. in the Red River was significantly lower than the canals. This may be because waste water is diluted in the river. In Vietnam,

monitoring fecal bacterial groups, especially Bacteroides group. with FISH method is useful to improve the bacterial water quality and avoid threats to public health from contaminated water.

References

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