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Spectrofluorometric Assay for Rapid Detection of Total and Fecal Coliforms from Surface Water

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With a spectrofluorometer, the length of the incubation time required in the fluorogenic assay was reduced to 12 h. The threshold emissions for reading the fluorogenic reaction by the spectrofluorometer were 5 and 10 U for lauryl tryptose broth media containing 4-methylumbelliferyl-β-D-galactoside and 4-methylumbelliferyl-β-D-glucuronide, respectively. These two kinds of threshold units were equivalent to known concentrations of free 4-methylumbelliferone of 2.5 and 6 μM, respectively, in lauryl tryptose broth media.

Enumeration of total and fecal coliforms, which are closely related to a bacterial group in the family *Enterobacteriaceae*, has been used as an indicator for fecal contamination of water samples for over 100 years (2). Traditional methods of detecting coliforms, however, require a long detection time of up to 96 h and complicated procedures (1). Recently, a simple and rapid method using fluorogenic substrates has been developed for detecting coliforms in surface water (3–5, 7, 9), drinking water (3–5, 8, 14), marine water (12) and snow (11), wastewater (9), fecal materials (15), and foods (10, 13). Total coliform bacteria have the ability to produce the enzyme β-D-galactosidase, which hydrolyzes 4-methylumbelliferyl-β-D-galactoside (MUgal). Fecal coliform bacteria or *Escherichia coli* can produce another enzyme, β-D-glucuronidase, which catalyzes the breakdown of 4-methylumbelliferyl-β-D-glucuronide (MUglu). Both enzymes release 4-methylumbelliferone (MU), a compound that fluoresces under long-wavelength (366 nm) UV light (6).

Previous fluorogenic assays have used long-wavelength UV light for reading the reactions, which is often subjective, requiring appropriate positive and negative culture controls for each experiment. Furthermore, test results show variations depending on the operators (13). Some recent studies recommended that the incubation time for reading fluorescence intensities with the long-wavelength UV lamp should be longer than 18 h unless a more sensitive instrument is used. The purpose of this study was to examine the efficiency of a spectrofluorometer in reading the fluorogenic reaction for rapid, specific, and sensitive detection of total and fecal coliforms from surface water.

A total of 95 river and 153 reservoir water samples were collected from the Han River and Lakes Soyang and Daechung in Korea from February to October 1993. The Han River is now used as the only raw water source of public water supply in the Seoul metropolitan area. Lake Soyang, located upstream of the Han River, is the largest artificial multipurpose reservoir in Korea and maintains an oligotrophic-to-mesotrophic state with a relatively clean water quality. However, blooms of cyanobacteria, mainly *Anabaena* spp., have been annually reported in some tributaries of the lake. Lake Daechung is the only multipurpose reservoir in Kum River, located in the central region of South Korea. An annual cyanobacterial bloom by some *Microcystis* species occurs, and thus the lakewater is highly polluted from late summer to early fall.

As a fluorogenic method, 100 mg (each) of MUgal (Sigma Chemical Co.) for total coliforms and MUglu (Sigma Chemical Co.) for fecal coliforms was added to 1,000 ml of lauryl tryptose broth (LTB) medium. Five serial dilutions described in the LTB-MPN method in *Standard Methods for the Examination of Water and Wastewater* (1) were inoculated in each 10 ml of fluorogenic LTB medium prior to incubation at 35°C. The fluorescence intensity was checked at 2-h intervals (4th through 12th h of incubation) with a Shimadzu RF-540 spectrofluorometer (excitation wavelength, 360 nm; emission wavelength, 460 nm; sensitivity, 2; ordinary scale, 4). The mean emissions of the fluorescence of the control tube were subtracted from the fluorescence intensities of the water sample tested.

The rates of recovery of total coliforms by the LTB-MPN method were 78% with 18 h of incubation and 82% with 24 h of incubation by gas production, but it conspicuously increased to 98% with 18 h of incubation by the fluorogenic method. Clark and El-Shaarawi (5) showed that fluorescent responses appeared more quickly than gas production and that fluorescence was detected earlier when incubated at 35°C than at 44.5°C. They also showed that only weak fluorescence was detected within 6 h, while much stronger fluorescence was detected only after 24 h of incubation by long-wavelength UV light. It was revealed by Feng and Hartman (9) that the enzymatic reaction of β-D-glucuronidase was rapid and reached the maximum level within 4 h, even though some weakly positive strains required further incubation up to 16 h.

Spectrofluorometry was utilized to improve the detection efficiency of fluorescence. The optimization process of spectrofluorometry was used to obtain the threshold value of fluorescence emission and the minimum incubation time required for detection of coliforms. Both LTB-MUgal and LTB-MUglu tubes showed an insignificant fluorescence emission of about 2 U even after a 10-h incubation, which was nearly the same emission scale as that shown in the control tubes that contained no coliforms. With 12 h of incubation, however, they showed distinct fluorescence emission levels of about 8 and 11 U in LTB-MUgal (Fig. 1a) and LTB-MUglu (Fig. 1b), respectively. The results show that incubation time can be reduced from 24 h with a visual method with UV light to 12 h by spectrofluorometry.

The next problem to be solved was to set up the threshold

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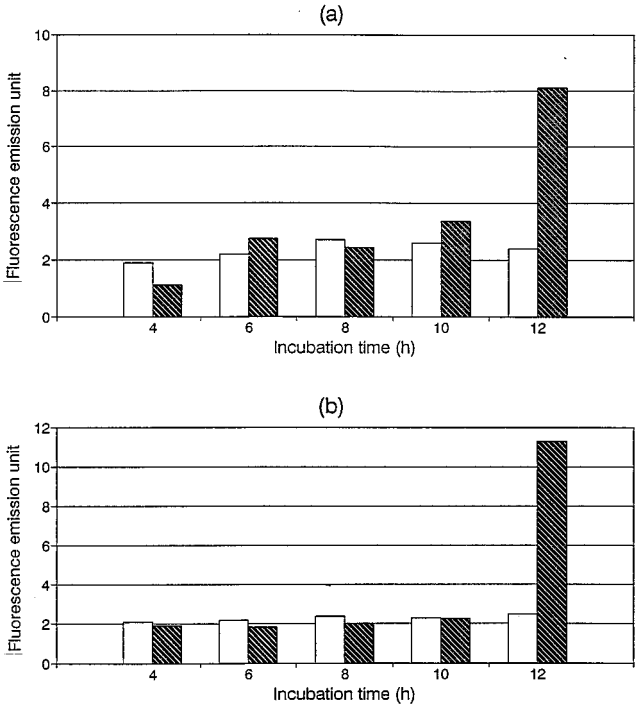

FIG. 1. Detectable fluorescence emissions of LTB-MUgal (a) and LTB-MUglu (b) with incubation time. Open bars, fluorescence negative; hatched bars, fluorescence positive.

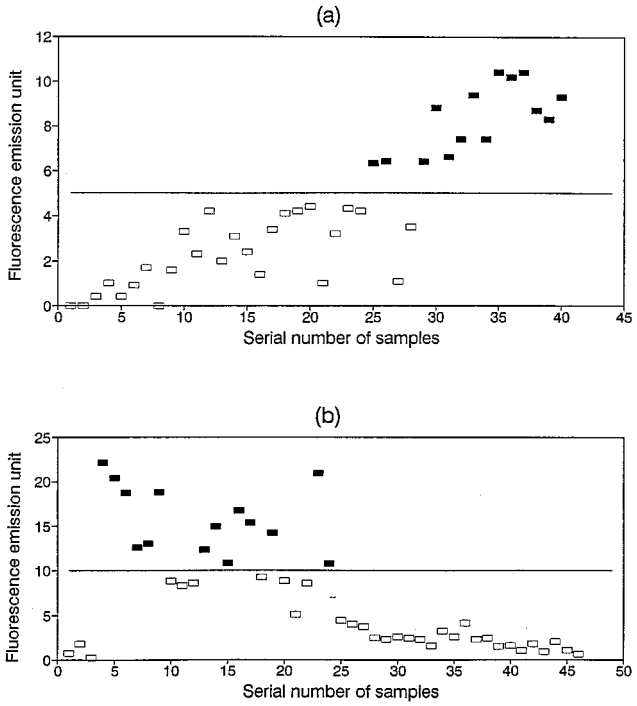

FIG. 2. Threshold fluorescence emissions of LTB-MUgal (a) and LTB-MUglu (b) for assessing the presence or absence of coliforms after 12 h of incubation. Open rectangles, fluorescence negative; solid rectangles, fluorescence positive.

emission unit to differentiate fluorescence-positive tubes from negative ones. Water samples which contained either only heterotrophic plate count bacteria or heterotrophic plate count bacteria with coliforms were randomly selected, and fluorescence was measured after 12 h of incubation by the spectrofluorometer. The boundary fluorescence units by which water samples tested were able to be grouped as coliform present or coliform absent were identified. However, the threshold units varied with the type of fluorogenic substrates. All of the coliform-absent LTB-MUgal tubes recorded less than 5 fluorescence emission units, but all of the coliform-present tubes recorded more than 5 fluorescence emission units (Fig. 2a). The threshold emission unit of LTB-MUglu was 10 (Fig. 2b). Therefore, LTB-MUgal tubes recording more than 5 emission units are considered total coliform positive, and LTB-MUglu tubes recording more than 10 emission units by spectrofluorometry are considered fecal coliforms. Spectrofluorometry could provide us with an objective reading of fluorescence and could significantly reduce subjective reading of the fluorogenic reaction. These threshold units were converted to micromolar concentrations of MU with a calibration standard curve of the spectrofluorometer readings versus standards for known free MU concentrations in LTB media. Five and 10 units were equivalent to 2.5 and 6 μM free MU in LTB media, respectively (Fig. 3). The rates of hydrolysis of both fluorogenic substrates were 1.0% for LTB-MUgal and 2.1% for LTB-MUglu.

The American Public Health Association (1) recommended EC media for detection of fecal coliforms. Nevertheless, in our assay, LTB-MUglu media was selected instead of EC-MUglu on the basis of the following results. For 100 water samples, EC-MUglu and LTB-MUglu were compared with EC-MPN with respect to an agreement rate, correlations by linear regression and Pearson analyses, and differences by paired *t* test. The agreement rates between EC-MPN and EC-MUglu and EC-MPN and LTB-MUglu were the same, 84%. The correlation coefficients (r^2) were 0.885 for the former and 0.917 for the latter relationships, and the *t* values were 0.00 for both. The results showed that both methods were significantly correlated with each other and that there were no differences between the two, other than the fact that LTB-MUglu showed a slightly higher correlation with EC-MPN than with EC-MUglu. The American Public Health Association (1) also recommended that fecal coliforms or *E. coli* be incubated at 44.5°C rather than at 35°C. In our assay, 87% of LTB-MUglu-positive tubes at 35°C fluoresced at 44.5°C and 80% of EC-MUglu-positive tubes at 35°C fluoresced at 44.5°C. This result shows that the

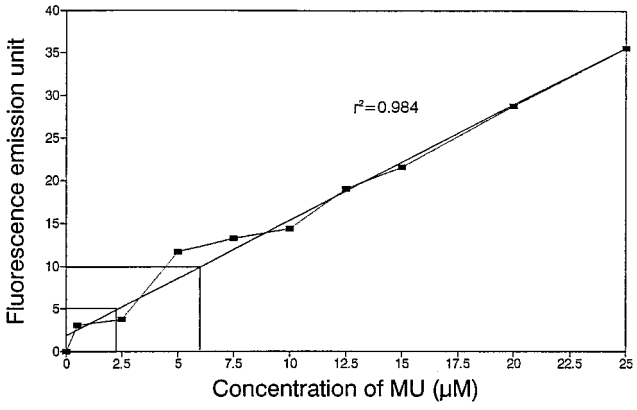

FIG. 3. Calibration standard curve (diagonal line) of free MU in LTB media measured by spectrofluorometer. Solid rectangles, units measured.

enzymatic reaction of fecal coliforms may be inhibited by high temperature. Clark and El-Shaarawi (5) supported this possibility by their study, in which more EC-MUglu tubes produced gas and fluorescence at 35°C than at 44.5°C.

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