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ORIGINAL PAPER

A rapid method for the detection of representative coliforms in water samples: polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA)

Jong-Tar Kuo · Chiu-Yu Cheng · Hsiao-Han Huang · Chia-Fen Tsao · Ying-Chien Chung

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Abstract Methods to detect the presence of coliform bacteria in drinking water usually involve a series of complex cultivating steps that are time-consuming and subject to external influences. For this reason, the new 16S rRNA probe has been developed in this study as an alternative detector PCR-ELISA technique that does not involve the culture of bacteria and that is able to detect, identify, and quantify the representative coliform species present in water samples. Our results indicate that this technique is both rapid (detection time of 4 h) and accurate (1.4% error rate). The limit of detection (LOD) was 5 CFU/100 ml for total coliforms, which meets the standards set by most countries for drinking water. Our comparative study demonstrated that this PCR-ELISA method is superior to current conventional methods in terms of detection time, LOD, and accuracy.

Keywords Coliforms · Drinking water · Enzyme-linked immunosorbent assay · Polymerase chain reaction

Introduction

Total coliforms and/or fecal coliforms by themselves are usually not pathogenic; they are indicator organisms, which means they may indicate the presence of other pathogenic bacteria in the water system [1]. Representative coliforms include *Escherichia coli*, *Citrobacter* spp., *Enterobacter aerogenes*, and *Klebsiella* spp., all of which are easy to detect and count [2]. A variety of methods for detecting total coliforms or fecal coliforms in water, such as multiple-tube fermentation, membrane filtration, immunofluorescence antibodies (IFA), chromo agar culture media test, and fluorescence in situ hybridization (FISH), have been developed [3–7]. Of these, multiple-tube fermentation and the membrane-filter method are the most commonly used and best standardized methods, but they involve the culture of the water samples and, therefore, have all of the disadvantages of culture systems. The former, which involves the fermentation of variously diluted water samples with culture medium (lauryl tryptose broth) and subsequent measurements of gas formed by the bacteria, is time-intensive, and the result is always an overestimation [4, 8]. In the membrane filter method, a standard volume of sampled water is filtered through a membrane that retains the microorganism(s) on the membrane surface. However, cross-contamination can easily occur during filtering, toxic material(s) causing analysis failure can accumulate on the filter, and very turbid water samples cannot be analyzed [2, 5]. These methods can be used with IMViC tests [9] to detect the presence of fecal or non-fecal coliforms (*E. coli, K. pneumonia, E. aerogenes*, or *C. freundii*). Chromo agar is a chromogenic culture media that allows instant pre-identification of eyes of colonies of *Candida, Salmonella, E.coli*, urinary tract pathogens, etc. However, it is frequently a time-consuming and challenging task [10].

The IFA assay is based on the detection of antigen–antibody interactions that are detectable with a fluorescence microscope [3]. It is difficult to prepare and purify the highly specific monoclonal antibodies necessary. The FISH methodology [7] involves the use of fluorescently labeled DNA probes for detecting or confirming gene or chromosome abnormalities that are generally beyond the resolution

J.-T. Kuo · C.-Y. Cheng · H.-H. Huang · C.-F. Tsao · Y.-C. Chung (✉)
Department of Biological Science and Technology, China University of Science and Technology, Taipei 115, Taiwan, ROC
e-mail: ycchung@cc.cust.edu.tw

of routine cytogenetic studies. However, again, specific probes must be constructed and fluorescently labeled. In addition, high densities of bacteria growth skew the measurement of the fluorescence, especially in environmental water samples.

The polymerase chain reaction (PCR) assay is currently one of the most sensitive, specific, and versatile laboratory detection tools. It can be used with the enzyme-linked immunosorbent assay (ELISA), which is also highly sensitive and specific [11] and has the added advantage of not requiring radioisotopes (radioactive substances) or a costly radiation counter. A combined PCR–ELISA assay is more sensitive than the PCR assay alone, and such a combined approach has been applied in detecting *Legionella pneumophila* in industrial cooling tower water [12], viral genomes [13], *Listeria monocytogenes* in food [14], and *Candida* spp. in patients with hematologic malignancies [15]. In addition, PCR–ELISA has been applied to detect *Escherichia coli* in milk and oysters [16, 17]. To date, however, there is no published report on its use for coliform limitation detection in 6 CFU/100 ml samples of drinking water [18].

We report here our development of a rapid detection method, based on PCR–ELISA technology, which is applicable for both identifying and counting coliforms (using *E. coli, K. pneumoniae, E. aerogenes* and *C. freundii* as representative coliforms) in water samples. The 16S rDNA of the four strains tested was first compared to identify a unique sequence for the specific probe, and then PCR–ELISA technology was applied for identification purposes. Simulated water samples were tested using traditional methods and the newly developed PCR–ELISA method to illustrate the feasibility of the PCR–ELISA approach for the rapid detection of coliforms.

Materials and methods

Bacterial strains

The strains of coliforms were kindly provided by the Bioresource Collection and Research Center (BCRC) in Taiwan. These included *E. coli* strain ATCC 23815 (serial no. BCRC 10314), *Klebsiella pneumoniae* ATCC9997 (serial no. BCRC), *Enterobacter aerogenes* ATCC 13048) (serial no. BCRC 10370), and *Citrobacter freundii* ATCC 8090 (serial no. BCRC 12291).

Probe design and primers

We used the BLAST program on the NCBI website to search for specific sequences of these strains and then constructed species-specific sequences for use as probes. The universal primer sequences of the 16S rDNA primer set used in the PCR were: 16SR, 5′-AGAAA GGAGG TGATC CAGCC-3′; 16SF, 5′-AGAGT TTGAT CCTGG CTCAG-3′.

Cell growth and PCR

The *E. coli, K. pneumoniae, E. aerogenes*, and *C. freundii* strains were cultured at 37°C in Luria–Bertani (LB) broth unless otherwise stated. Growth of the bacterial cultures was periodically determined by measuring the absorbance at an optical density of 600 nm (OD_{600}) on a Shimatzu UV-1201 spectrophotometer. PCR amplification was carried out in a reaction mixture containing the genomic DNA of the respective bacterial strain (0.3 ng), the 16S rDNA primer set (100 ng/μl), dNTPs (0.2 mM), and Taq DNA polymerase (1 U). The PCR cycling program consisted of denaturation at 94°C for 2 min; 25–35 amplification cycles of 94°C for 30 s, 55–63°C for 30 s, and 68°C for 60 s; a final 5-min extension step at 72°C. All PCR products and a 100-bp ladder (New England Biolabs, Beverly, MA) were run on a 1.5% agarose gel (SeaKem GTG agarose, BMA, Rockland, ME) and stained with ethidium bromide (EtBr) [19].

Optimization of the DNA preparation

The DNA was prepared using the commercially available QiAamp Tissue kit (Qiagen AG, Basel, Switzerland). When the cell number was lower than 10,000 CFU, we used the traditional extraction method (i.e., harvested total cells by centrifuge and used PCR buffer to lysis cells) to extract total genomic DNA from bacteria. The amount of genomic DNA harvested was measured in serially diluted suspensions of *E. coli, K. Pneumoniae*, *E. aerogenes*, and *C. freundii* cells (1) by loading an aliquot of the genomic DNA on an agarose gel, running the samples in the electrophoretic system, and comparing the resulting bands with those bands of known concentrations of a DNA marker; (2) after PCR amplification of an aliquot of each DNA extract and determination of the highest dilution that produced a visible band by agarose gel electrophoresis.

Detection of PCR products by ELISA

The PCR products (4–20 μl containing 7–176 μg DNA/ml) were mixed with 20 μl of a biotin-labeled probe (20 pmol), heated at 95°C for 5 min, and then incubated at 50–55°C for 5 min for annealing. After the reaction mixture had cooled to 4°C for 1 min, 200 μl of blocking buffer [0.05% phosphate-buffered saline (PBS), 0.1% Tween-bovine serum albumin (BSA)] was added and the reaction mixture and transferred to the well of a streptavidin-coated microtiter strip (Roche, Indianapolis, IN) for 30 min at 37°C.

Following washes with PBS 0.1% Tween, 200 µl of working solution of conjugated anti-digoxigenin-peroxidase Fab fragments (150 U/vial; Roche) was added to each well and the plate incubated at 37°C for 60 min. The optimal working solution was titrated, and the results ranged from a 1:10,000 to a 1:14,000 dilution from the stock solution. After four washes and the addition of 200 µl of 3,3′,5′, 5-tetramethylbenzidine substrate for 5–30 min (Sigma–Aldrich, St. Louis, MO), the reaction was stopped with the addition of 100 µl 0.5 M H_2SO_4. The OD value was read at 450 nm in an ELISA plate reader [20].

Calibration curves and limit of detection of the PCR–ELISA method for various strains

To evaluate the relationship between cell number and PCR–ELISA value, Table 2 shows a series of regression calculations of total DNA concentrations vs. cell numbers and PCR–ELISA, demonstrating the capability of the PCR–ELISA technique to detect the existence of coliform cells. Also, to establish the relationship between cell numbers and bacterial optical density, we compared the number of *E. coli, K. pneumoniae, E. aerogenes*, and *C. freundii* cells counted in serial dilutions plated on LB agar and the absorbance of the dilutions at OD_{600}. To establish the relationship between the total genomic DNA concentrations of each strain and its cell numbers, total genomic DNA of these strains was extracted and purified following the process described in the section about optimization of the DNA preparation. Finally, to establish the relationship between the concentration of the genomic DNA and that of the PCR products, we measured the concentrations of PCR products. Based on the results of these analyses, we were able to construct calibration curves between (1) cell numbers and concentrations of PCR products of each representative coliform species and (2) cell numbers and PCR-ELISA values for each representative coliform species. The minimal but visible PCR–ELISA signals allowed us to determine the LOD for detecting these coliforms. All these results from regression equations, LOD, and standard error were measured by repeating them three separate times.

Detection of these representative coliforms in the simulation water samples

Due to the abundance of naturally occurring factors in water that can interfere with the detection of coliforms, our research was a simulation in which tap water was used to evaluate the feasibility of our PCR-ELISA method for detecting representative coliforms. The tap water was autoclaved and mixed with selected concentrations of coliforms (320 CFU/ml *E. coli,* 360 CFU/ml *K. Pneumoniae*, 400 CFU/ml *E. aerogenes*, and 420 CFU/ml *C. freundii*). The bacteria genomic DNA was then extracted from the simulation water samples as the template in the PCR-ELISA experiments. Using empirically determined calibration curves, we estimated the cell numbers of coliforms in the simulation water samples; other conventional methods, such as the plate count method, multiple-tube fermentation method, and membrane filtration method, were used to provide estimates for comparison.

Results and discussion

Optimum conditions in detecting representative coliforms by the PCR method

The specific sequences used as probes for each 16S rDNA of each of the coliforms tested in our study were checked in control experiments (data not shown) and subsequently used in PCR-ELISA methods to identify these strains. The probe sequences in *E. coli, K. pneumoniae, E. aerogenes*, and *C. freundii* were GAGTAAAGTTAATACCTT TGCTC (16S rDNA target position 454–476), CGGTG AGGTTAATACCTCATCGA (16S rDNA target position 426–449), TTGCGGGAC TTAACCCAACATTTC (16S rDNA target position 426–449), and CGTTGTGG TTAATAAC (16S rDNA target position 435–457), respectively. The probe specificity was carefully checked using Southern blotting (data not shown). Two probes have been adapted for the detection of *Enterobacteriaceae* in general: Mittelman et al. [21] constructed the ENTERO probe for testing for urinary tract infection, and Loge et al. [22] constructed the ENT1 probe for testing wastewater. However, these two probes are incapable of specifically detecting other coliform strains. In contrast, the COLINSINTU probe was developed specifically for detecting *E. coli* [23]. In terms of 16S rDNA position, these probes have different sequences than those that we constructed for our study. Accordingly, the probes for coliform 16S rDNA used in the PCR–ELISA technique were first demonstrated and established in this research.

To estimate the total DNA concentration, bacterial cells growing in culture were first harvested in the mid-log phase by centrifugation. Total DNA of *E. coli, K. pneumoniae, E. aerogenes*, and *C. freundii* was then measured by DNA density analysis software (Bio-Rad analysis system; Bio-Rad, Hercules, CA), with approximately 34 ± 1.8, 30 ± 0.9, 32 ± 1.5, and 35 ± 2.1 ng/µl, respectively. Recent studies indicated that the annealing temperature and number of reaction cycles in the PCR program may significantly affect the concentration of DNA in the PCR products [24]. Accordingly, we tested various annealing temperatures (55, 58, 60, and 63°C) using 1% of the total genomic DNA reaction mixture as the PCR template, resulting in 60°C as the

Table 1 Concentrations of PCR products at three different PCR reaction cycles for representative coliforms at an annealing temperature of 60°C

PCR reaction cycles	DNA concentration (ng/μl)			
	E. coli	*K. pneumoniae*	*E. aerogenes*	*C. freundii*
25	35 ± 1.7	37 ± 1.8	59 ± 2.7	38 ± 2.1
30	59 ± 2.7	59 ± 2.9	74 ± 3.5	64 ± 3.1
35	53 ± 3.2	50 ± 3.4	68 ± 3.8	61 ± 3.0

optimal annealing temperature (data not shown). Thus, the PCR program in this study with the various reaction cycles (25, 30, and 35 cycles) has been changed into the verified annealing temperature of 60°C. Table 1 shows the effect of various numbers of annealing cycles on the total PCR product per coliform strain, as determined by the Bio-Rad analysis system. The highest concentration of PCR product was obtained using 30 reaction cycles, with DNA concentrations in *E. coli, K. pneumoniae, E. aerogenes*, and *C. freundii* of 59 ± 2.7, 59 ± 2.9, 74 ± 3.5, and 64 ± 3.1 ng/μl, respectively. Accordingly, the number of PCR reaction cycles was designated to be 30 in this study.

Regression relationships among different parameters in representative coliforms

Table 2 presents the regression relationships among the different parameters determined for the coliforms tested here. Knowing the relationship between total DNA concentration and cell number as well as that between total DNA concentration and PCR product concentration, we were able to obtain the relationship between cell number and PCR product concentration. After establishing the relationship of total DNA concentrations and PCR–ELISA signals, we were also able to obtain the relationship between cell numbers and PCR–ELISA signals. Figure 1 shwos the calculation of these relationships in *E. coli.* In this case, the regression equations for *E. coli, K. pneumoniae, E. aerogenes*, and *C. freundii* were $y = 0.0005x + 0.1535$, $y = 0.0005x + 0.1408$, $y = 0.0006x + 0.1394$, and $y = 0.0006x + 0.1111$, respectively. All of the R^2s in the regression analysis of these relationships in the representative coliforms were >0.94, indicating that these regression relationships were credible ($P = 0.05$).

Limit of detection in PCR and PCR–ELISA for representative coliforms

Figure 2 shows the effect of serial dilutions on the LOD of the electrophoresed PCR products. Introducing these concentrations into the regression equation between the cell numbers and concentrations of PCR products listed in Table 2, we were able to estimate that the LODs for detecting *E. coli, K. pneumoniae, E. aerogenes*, and *C. freundii* were 128, 97, 125, and 116 CFU/100 ml, respectively. Results of LOD and standard error were calculated by repeating them three separate times. Ragnault et al. [23] developed the COLINSINTU probe to detect *E. coli* in urine, water, and food samples using FISH methodology. Although the COLINSINTU probe has exhibited good specificity, it is still difficult to identify other coliforms that coexist in drinking water using this method. Our methodology of detecting coliforms using PCR coupled with specific probes, the COLINSINTU probe with FISH, and the multiplex real-time PCR method all meet the LOD requirement set down in general regulations, such as those in effluent standards for wastewater and the quality standard for drinking water sources; however, they cannot meet the LOD requirement set down for the drinking water standard (total coliforms <6–10 CFU/100 ml) of most countries [18]. Thus, our PCR detection method needs to be improved to satisfy the LOD requirement for the drinking water standard.

Table 2 Regression analysis relationships among different parameters in coliforms

Conditions	Coliform strains			
	E. coli	*K. pneumoniae*	*E. aerogenes*	*C. freundii*
Log total DNA concentrations (x) versus cell numbers (y)	$y = 19763e^{1.2284x}$ $R^2 = 0.9654$*	$y = 20204e^{1.1908x}$ $R^2 = 0.9855$*	$y = 13099e^{1.186x}$ $R^2 = 0.9598$*	$y = 11593e^{1.1626x}$ $R^2 = 0.9405$*
Log total DNA concentrations (x) versus PCR products (y)	$y = 100.28e^{0.0785x}$ $R^2 = 0.9492$*	$y = 100.28e^{0.0785x}$ $R^2 = 0.9426$*	$y = 114.81e^{0.1815x}$ $R^2 = 0.9469$*	$y = 130.95e^{0.0916x}$ $R^2 = 0.9483$*
Cell numbers (x) versus PCR products (y)	$y = 0.0189x + 68.042$ $R^2 = 0.9519$*	$y = 0.0223x + 89.394$ $R^2 = 0.9813$*	$y = 0.0494x + 44.217$ $R^2 = 0.9897$*	$y = 0.0332x + 84.146$ $R^2 = 0.9713$*
Log total DNA concentrations (x) versus PCR-ELISA (y)	$y = 0.0966x + 0.8681$ $R^2 = 0.9711$*	$y = 0.0842x + 0.7682$ $R^2 = 0.9858$*	$y = 0.0808x + 0.7383$ $R^2 = 0.9731$*	$y = 0.0587x + 0.5709$ $R^2 = 0.9852$*
Cell numbers (x) versus PCR-ELISA (y)	$y = 0.0005x + 0.1535$ $R^2 = 0.9641$*	$y = 0.0005x + 0.1408$ $R^2 = 0.9435$*	$y = 0.0006x + 0.1394$ $R^2 = 0.9436$*	$y = 0.0006x + 0.1111$ $R^2 = 0.9439$*

*Level of significance for all of the regression square is $P < 0.05$

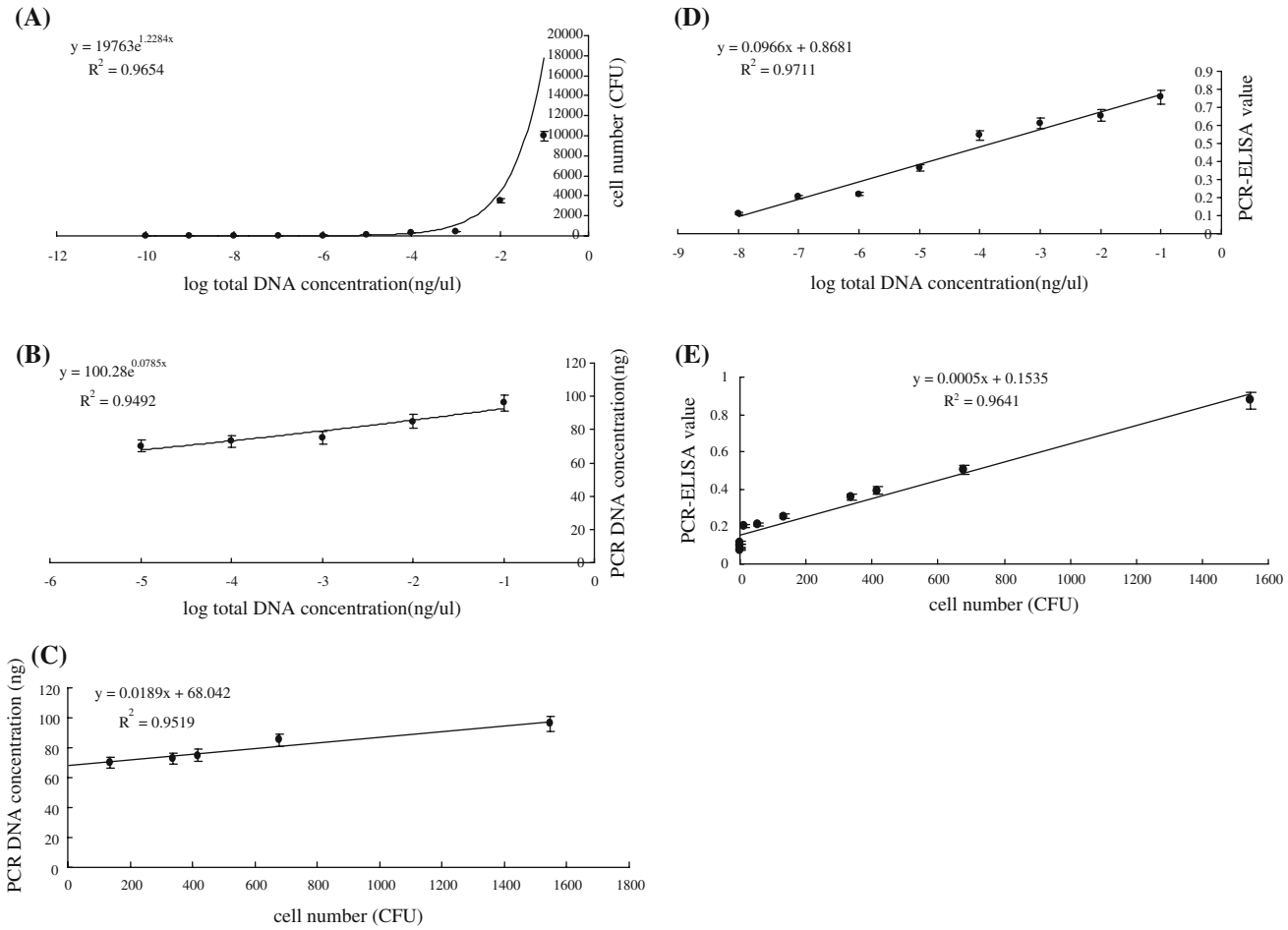

Fig. 1 The regression analysis relationships among different parameters in *E. coli.* **a** Log total DNA concentrations vs. cell numbers; **b** log total DNA concentrations vs. PCR products; **c** cell numbers vs. PCR products; **d** log total DNA concentrations vs. PCR–ELISA; **e** cell numbers vs. PCR–ELISA

To improve the LOD for detecting representative coliforms, we adapted the PCR technique to one of PCR–ELISA, which involves amplification of the bacterial DNA by multiplex PCR and subsequent hybridization of the PCR product to specific oligonucleotide probes in an ELISA-based format. The result may be an increase in the sensitivity of the detection system [25]. The first PCR product was coated on the plate and hybridized with a biotin-labeled specific probe for each coliform strain. To evaluate the effect of probe concentration on the LOD, we evaluated the specific probe at concentrations of 1 or 10 μM and found that a probe concentration of 10 μM was relatively more efficient for all four coliform strains (Table 3). The LODs at probe concentrations of 10 and 1 μM for *E. coli* were 1 and 5.7 CFU/100 ml, respectively, when these PCR–ELISA signals were introduced into the regression equations in Table 2. In addition, the LOD of the method using 10 μM of specific probes was 1, 1, 2, and 1 in *E. coli*, *K. pneumoniae, E. aerogenes*, and *C. freundii*, respectively. The LOD in total coliform detection by using specific 16S rRNA probes was 5 CFU/100 ml, which does satisfy the drinking water standard. The other use of PCR–ELISA technology to detect *E. coli* in milk and oysters has been reported by Gonzalez et al. and Daly et al. [16, 17]. In addition, the probe genes for detecting *E. coli* in milk and oysters were *alr* and *lamB* gene, with LODs of 100 CFU/ml and $10–10^5$ CFU/g, respectively [16, 17]. However, to satisfy the drinking water standard, LOD has to correspond with the limit of less than 6 CFU/100 ml. We therefore conclude that the PCR–ELISA method is not only efficient in detecting different species, but it can also be used to assess cell numbers in drinking water.

Using PCR–ELISA to detect representative coliforms in the simulation water samples

Although relatively high concentrations of coliforms are often present in polluted tap water, a number of substances in tap water may affect the sensitivity of the method to detect the coliforms. We therefore evaluated the feasibility

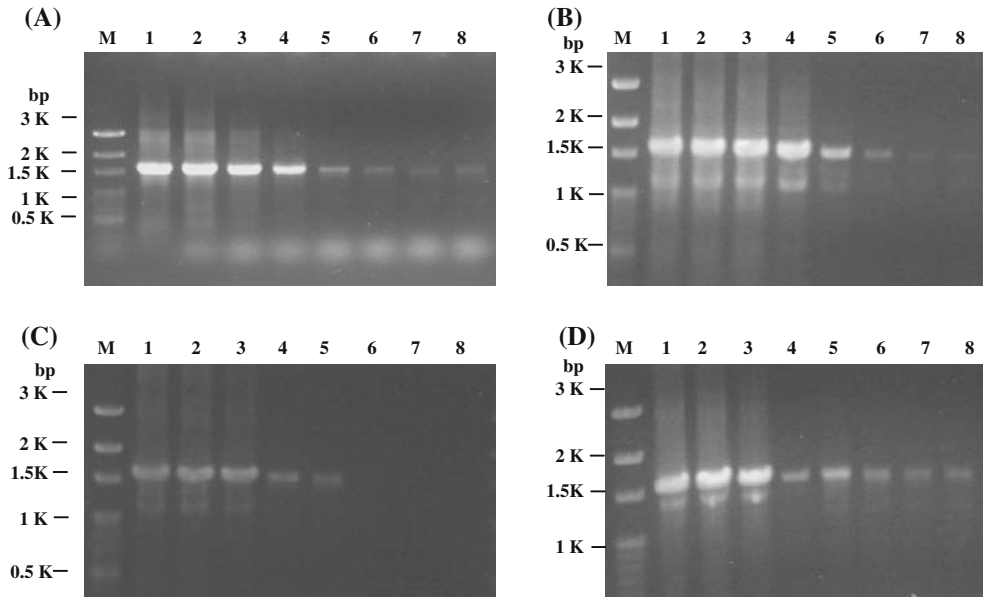

Fig. 2 The DNA electrophoresis of PCR reaction using 16S rDNA primers for representative coliforms. *Lanes 1–8*: PCR products when the solution containing the PCR products was diluted from 10^{-1} to 10^{-8}, respectively; M: marker. **a** *Escherichia coli*, **b** *Klebsiella pneumoniae*, **c** *Enterobacter aerogenes*, **d** *Citrobacter freundii*

Table 3 PCR-ELISA signals of the representative coliforms at different dilutions of the PCR products

Strains	Probe concentrations (μM)	PCR product dilution									
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
E. coli	10	0.876	0.503	0.392	0.356	0.253	0.212	0.203	0.114	0.098	0.076
	1	0.739	0.437	0.396	0.329	0.246	0.203	0.186	0.097	0.019	0.01
K. pneumoniae	10	0.952	0.542	0.411	0.376	0.256	0.216	0.2	0.109	0.043	0.032
	1	0.657	0.501	0.388	0.302	0.221	0.199	0.183	0.092	0.035	0.016
E. aerogenes	10	0.903	0.542	0.386	0.345	0.278	0.206	0.157	0.1	0.064	0.034
	1	0.595	0.475	0.356	0.313	0.243	0.196	0.14	0.083	0.057	0.016
C. freundii	10	0.851	0.42	0.323	0.283	0.227	0.203	0.146	0.105	0.02	0.012
	1	0.492	0.383	0.288	0.21	0.19	0.176	0.129	0.088	0.015	0.010

of our PCR–ELISA technique for detecting representative coliforms in the simulation tap water samples and compared these results with those obtained using the plate count method, multiple-tube fermentation method, and membrane filtration method. The results of this comparison are shown in Table 4. Among the coliform detection methods tested, the multiple-tube fermentation method was the most time-intensive and had the highest error rate (13.3%). Although the membrane filtration method was relatively better in terms of detection time and error rate (5.3%), we used this method in an aseptic environment, and it is likely that its error rate would increase under less stringent conditions. Davies and Apte [26] developed a fluorimetric assay method to determine FC and obtained a LOD of 300 FC/100 ml in a measuring interval of 1 h and a 2% error rate compared to a standard membrane filtration procedure. Georges et al. [27] developed an enzymatic method to detect FC and total coliforms, and Fiksdal and Tryland [28] subsequently found that the LODs of the enzymatic method were 20 CFU/100 ml for FC and 340 CFU/100 ml for total coliforms. They concluded that the enzymatic method is a good method for identifying FC or non-FC coliform groups, but that the LOD must be improved. Rapid enzyme assay techniques based on direct measurement of β-D-galactosidase (GALase) activity without selective cultivation are used for rapid estimation of the level of coliform bacteria and *E. coli* in water samples. Such techniques perform rapid detections; however, enzyme activities and

Table 4 Comparison of the different detection methods in detecting representative coliforms

Detection methods	Detection time	Cell numbers in different strains (CFU/ml)				Total cell numbers (CFU/ml) 1,500 CFU/ml	Error rate[a] (%)
		E. coli 320 CFU/ml	*K. pneumoniae* 360 CFU/ml	*E. aerogenes* 400 CFU/ml	*C. freundii* 420 CFU/ml		
Plate count method[b]	24 h	–	–	–	–	1,546 ± 12	3.1 ± 0.8%
Multiple-tube fermentation method	4 day	–	–	–	–	1,700 ± 15	13.3 ± 1%
Membrane filtration method	24 h	–	–	–	–	1,580 ± 18	5.3 ± 1.2%
PCR–ELISA	4 h	315 ± 2	350 ± 3	382 ± 5	432 ± 6	1,479 ± 16	1.4 ± 1.1%

[a] Error rate was the percentage of comparing cell number from detection method with actual coliform bacteria added

[b] Cultured with standard strains on LB agar plate

LOD should be deliberately considered beforehand. The aim of our study was to use PCR technology to amplify the specific DNA and the ELISA method to increase the sensitivity. This combined method required 4 h to detect the representative coliforms, and the error rate was only 1.4%. The PCR–ELISA method was also able to accurately identify each species of the representative coliforms tested, and the LOD in total coliform detection was only 5 CFU/100 ml, which clearly meets the drinking water standard. Based on these results, we suggest that our newly developed PCR–ELISA method shows promise for detecting coliforms in environmental water samples. Future studies in our laboratory will be directed towards evaluating the feasibility of this method for detecting coliforms in environmental water samples from various water bodies.

Conclusions

We have designed specific sequences as probes for detecting the presence of representative coliforms in water samples, including *E. coli*, *K. pneumoniae, E. aerogenes*, and *C. freundii*. We also established the optimum experimental conditions for running the PCR and PCR–ELISA analyses. Under these conditions, the representative coliforms in the simulation water samples could be identified within 4 h. Our results demonstrate that this PCR–ELISA method has a higher specificity and is more sensitive and more accurate than standard methods currently being used. This relatively short detection time mirrors the fast feedback nature of the PCR–ELISA in detecting coliforms. The LOD of 5 CFU/100 ml that we achieved with this method is superior to the standard set by most countries in their quality guidelines for drinking water standard (6–10 CFU/100 ml). Based on these results, we suggest that this PCR–ELISA method has the potential to become one of the better—if not the best—method for detecting coliforms in water samples.

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