Comparison of total and faecal coliforms as faecal indicator in eutrophicated surface water

J.E. Park*, T.S. Ahn, H.J. Lee*** and Y.O. Lee***

*Dept of Life Science, Daegu University, Kyung-San, 712-714, Korea (E-mail: *ecolomi@daegu.ac.kr*)

**Dept of Environmental Science, Kangwon National University, Chunchon, 200-701, Korea

***Dept of Life Science, Dong-A University, Busan, 604-714, Korea

Abstract The aim of this study was to evaluate the use of total coliforms (TC) and faecal coliforms (FC) using a membrane filtration method for precise monitoring of faecal pollution in Korean surface water. The samples were collected in Korea from both main rivers and their tributaries. Presumptive TC · FC were enumerated. The ratios of presumptive FC to TC were not constant, but varied widely, and TC were difficult to enumerate because of overgrowth by background colonies. For FC this was not the case. Seven hundred and three purified strains of presumptive TC · FC and their background colonies were biotyped using API 20E. Among 272 presumptive TC, non-faecal related species, *Aeromonas hydrophila* dominated (34.6%) and *E. coli* accounted for only 5.1%. In contrast, *E. coli* made up 89% of the 209 presumptive FC. Furthermore, of 164 background colonies on Endo Agar LES, 54.9% was *A. hydrophila,* while background colonies on m-FC Agar were few (58 strains), and despite their atypical colony appearance, most of them were biotyped as enteric bacteria. These results reveal that the detection of FC rather than TC using m-FC Agar is more appropriate for faecal pollution monitoring in eutrophicated surface water located in a temperate region.

Keywords Background colonies; eutrophicated surface water; faecal coliforms; faecal pollution monitoring; overgrowth; total coliforms

Water Science & Technology **Vol 54 No 3 pp 185–190**

Introduction

Total coliforms (TC) including *Escherichia coli* are used world-wide as an indicator for faecal pollution since Smith and Rodet suggested in 1893 that *E. coli* containing 10^5–10^9 cells/g faeces might be a useful indicating organism for faecal contamination (Kavka, 1978). Also, about 90 years ago, Gärtner postulated that *Vibrio cholerae*, *Shigella dysenteriae, Salmonella* sp. might have a high possibility of being present where *E. coli* was detected (Gärtner & Reploh, 1964). Since that time, the value of TC, and particularly *E. coli*, because of their ease of detectability, has been confirmed and re-evaluated by many water hygienists (Daubner 1972; Kohl, 1975; Edberg *et al.*, 2000; Leclerc *et al.*, 2001) to assess the microbiological safety of surface water and drinking water. However, TC have limited use as indicators of faecal pollution because TC may multiply in warm, tropical climates (Kavka, 1978; Lavoie, 1983). Moreover, the frequently occurring overgrowth phenomenon by background colonies on its isolation agar could lead to false monitoring of faecal pollution (Burlingame *et al.*, 1984; Franzblau *et al.*, 1984; Brenner *et al.*, 1993; Lee, 1996). The aim of this study was to re-evaluate water quality monitoring programmes based on quantitative TC, compared with those of FC for Korean surface water utilised for all purposes including as a drinking water source. Furthermore, a part of isolated TC/FC was differentiated using the API 20E test system to compare the proportion of *E. coli* to presumptive TC with those of FC, because *E. coli* is regarded as the best biological drinking water indicator for public health protection (Edberg *et al.*, 2000).

doi: 10.2166/wst.2006.467

Materials and methods

Sampling locations

The samples were collected aseptically in 250 cm^3 glass bottles from a water depth of 30 cm of four Korean main rivers (Han river, Nak-Dong river, Gum river and Young-San river) and their tributaries in the warmer months from April 2004 to August 2005.

Isolation of TC·FC and correlation between TC and FC

According to the Korean Standard method, TC both on m-Endo Agar or m-Endo Agar LES, and FC on m-FC Agar were isolated concurrently. Cellulose nitrate membrane filters (45 mm diameter, 0.45 μm pore size; Sartorius, Germany), were used for membrane filtration techniques. To obtain the relevant results, all water samples were performed in three serial decimal dilutions with five replicates. After culturing for 24 ± 2 h TC in a dry incubator at 35 °C and FC in a water bath at 44.5 °C, metallic sheen colonies, regarded as presumptive TC on Endo Agar and Endo Agar LES, and dark blue

Table 1 Seasonal fluctuation of TC and FC in Korean main rivers with their differently eutrophicated tributaries

Site	Han river		Nak-Dong river		Young-San river		Gum river	
Date	TC	FC	TC	FC	TC	FC	TC	FC
	CFU/100 mL		CFU/100 mL		CFU/100 mL		CFU/100 mL	
Apr. 04/05	717	0	270	0	180	2	1033	220
	6,150	0	24,000	11,000	1626	40	182	15
	5,180,000	232,400	637	13	162	0	1100	248
	1,073,000	15,600	145	30	165	0	1133	193
	4,703,000	7,000	6800	95	1600	0	4680	25
May. 04/05	61,500	950	2,915	54	23400	140	38	0
	222,500	15,750	31,666	4,033	2	0	2000	15
	8,633,000	2,000	150	30	365	136	9091	0
	27,450,000	10,150	1,600	30	4478	140	75758	610
	14,350,000	40,000	600	100	200	29	2780	38
Jun. 04/05	171,667	1,567	46,200	6	3160	40	1600	86
	177,750	1,933	106,888	555	16000	1560	1020	2
	11,500,000	24,500	4,300	30	16	0	878	220
	32,400,000	515,000	14,200	20	1211	337	2422	31
	19,900,000	273,333	30,000	4	7235	1739	1393	143
Aug. 04	3,500,000	363	1,850	96	2810	2500	1578	193
	2,600,000	490	22,944	1,822	61200	12650	4680	25
	13,366,667	2,133	7,000	98	2400	288	38	0
	20,050,000	1,833	44,000	1,180	244	0		
	16,650,000	5,600	430	9	23778	1800		
Sept.04	1,933,333	243	62,444	11,162	6015	1700		
	1,400,000	217	1,400	2	225	10		
	9,466,667	1,267	3,000	20				
	18,836,667	1,867	62,444	6				
	10,800,000	14,667	4,208	9				
Apr. 05			1400	2				
			3000	20				
			1208	87				
			0	0				
			2	10				
Jun. 05			1063	2				
			30500	11400				
			20500	1650				
			58000	5100				
			34000	2500				

TC: total coliforms, FC: Faecal coliforms

Table 2 Correlation between TC and FC obtained from four Korean main rivers and their tributaries in warmer months 2004–2005

Site	Han river	Nak-Dong river	Young-San river	Gum river
R	0.538**	0.464**	0.893**	0.776**
p-value	0.005	0.005	0	0
N	25	35	22	18

r: correlation coefficient; *p*-value: statistical probability; N: number of probe, $^{**}p < 0.01$

colonies, regarded as presumptive FC on m-FC Agar, were enumerated. The results were expressed as the mean value of five replicates in CFU/100 mL. Statistical analyses to evaluate the correlation between TC and FC were performed using the SPSS (Statistical Package for the Social Science, SPSS Inc., USA).

Species spectrum of presumptive TC·FC

For further differentiation of the presumptive TC·FC and background colonies, colonies were re-cultured once on the isolation medium and subsequently to nutrient agar and incubated for 24 h. Purified strains were subsequently characterised by the biochemical API 20E test system (Biomerieux, France) according to the manufacturer's instructions to analyse their species-spectrum.

Results and discussion

Comparison of TC and FC

The number of presumptive TC and FC were isolated from four main Korean rivers and their tributaries are summarized in Table 1. The counts of TC and FC were carried out only based on the morphology of colonies. As seen in Table 1, TC were generally one to four orders higher than FC. This difference tends to increase when water temperature as in the range of 18–27 °C and BOD_5 exceeded 20 mg/L in shallow tributaries (data not shown). In one sample FC was higher than TC in the same water volume (see Table 1; April 2005 of Nak-Dong river). This may have been due to overgrowth of TC by background colonies, especially on m-Endo Agar. Background growth became worse after heavy rainfall, especially in lower streams and shallow tributaries. Under these conditions, the TC counts may not reflect the real state of faecal pollution. Still, the TC counts determined in the four rivers and their tributaries correlated with FC counts (Table 2). The correlation between TC and FC in all samples from rivers as considered significant ($P < 0.01$).

Species-spectra of presumptive TC and FC

In total 703 purified strains of presumptive TC · FC and their background colonies were biotyped using the API 20E test system: 272 purified strains of presumptive TC isolated on m-Endo-Agar or Endo Agar LES and 209 purified strains of presumptive FC isolated on m-FC-Agar were biotyped using API 20E (Figure 1). Among the 272 metal sheen colonies, also presumptive TC, *E. coli* accounted for only 5.1%, while non-faecal species (*Aeromonas hydrophila*: 34.6%, *Pseudomonas* sp.: 6.3%) made up the major part (40.9%), followed by *Klebsiella* sp. (19.5%) and *Enterobacter* sp. (14.7%), species that belong to the coliforms, but whose occurrence is not uniquely connected with faeces (Edberg *et al.*, 2000; Leclerc *et al.*, 2001). In contrast, most strains of presumptive FC were identified as *E. coli* (89%) (Figure 1).

Of the 164 background colonies grown on m-Endo Agar and m-Endo Agar LES at 35 °C, 54.9% was identified as *A. hydrophila* 54.9% (Figure 2). This result implies

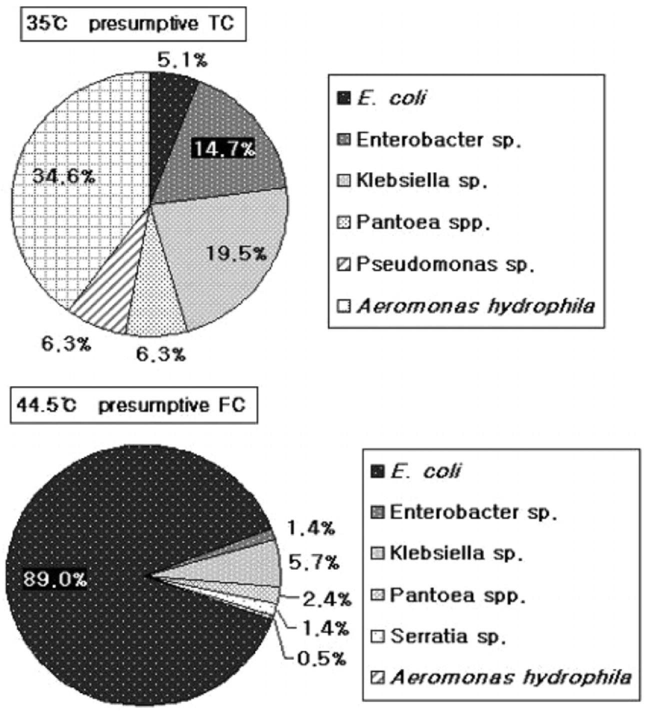

Figure 1 Species-spectra of presumptive total coliforms (TC) isolated on m-Endo Agar/Endo Agar LES at 35°C (above) and those of faecal coliforms (FC) on m-FC Agar at 44.5°C beneath

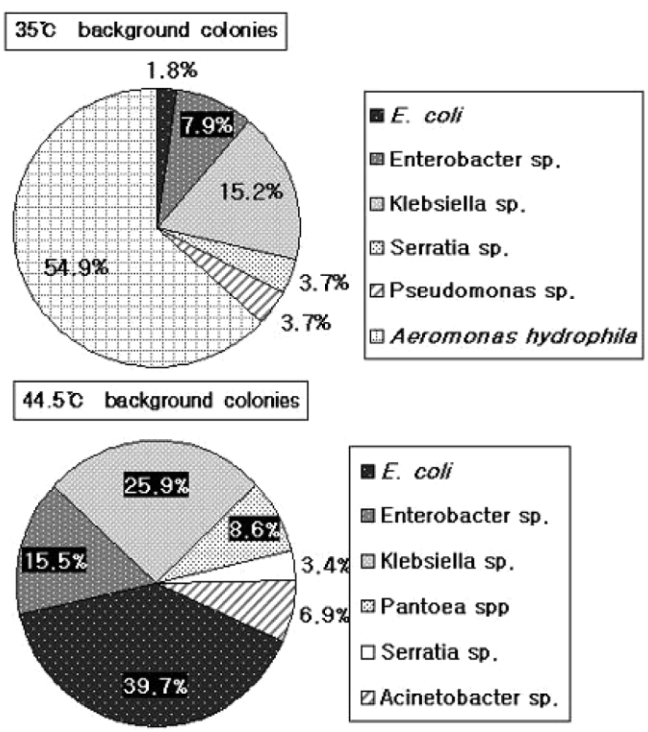

Figure 2 Species-spectra of background colonies grown on m-Endo Agar/m-Endo Agar LES at 35°C (above) and those of background colonies on m-FC Agar at 44.5°C beneath

that the majority of colonies grown on m-Endo-Agar or Endo Agar LES belongs to *A. hydrophila*, which are frequently isolated in water, especially in warmer months (Hazen *et al.*, 1978; Lee, 1986; Cavari *et al.*, 1981) irrespective of the presence of metal sheen colonies. In contrast, on m-FC Agar there were a few background colonies, maybe less than 1% of all colonies, and the 58 strains that were biotyped consisted of strains of enteric bacteria genera such as *E. coli* (39.7%), *Klebsiella* sp. (25.9%), *Enterobacter* (15.5%) and *Pantoea* sp. (8.6%). This result suggests that the dominating strain made of background colonies on m-Endo Agar, *A. hydrophila* was completely inhibited by elevation of the incubation temperature (44.5 °C). The interference of *A. hydrophila* with coliform detection has been demonstrated in a number of other studies (Burlingame *et al.*, 1984; Franzblau *et al.*, 1984; Lee, 1996). It implies that the overgrowth problem by background colonies resulting in the detection of TC could be solved when FC is determined for faecal contamination monitoring in surface water located in a temperate region such as Korea.

Conclusions

For faecal pollution monitoring in surface water located in a temperate region (Korea), both TC and FC were determined using membrane filtration techniques. The detection of TC may not be ideal for surveillance of faecal contamination in surface water, owing to the following observations from this study:

(1) The selective medium for TC was often overgrown by non-faecal related colonies, so-called background colonies, leading to false-positive results, and thus the obtained results for TC did not reflect the present faecal pollution state, especially in shallow tributaries during the warmer months.

(2) Among the enumerated TC and FC, 481 strains were purified and biotyped using the API 20E test system; *E. coli* accounted only for 5.1% of TC (272 strains) and non-faecal related species made up the major part of them (40.9%), whereas most presumptive FC was *E. coli* (89%), the species regarded as the best faecal indicator.

Therefore, it can be concluded that the detection of FC rather than TC using m-FC Agar might be reasonable for faecal pollution microbiological monitoring in eutrophicated surface water of temperate regions.

References

Brenner, K.P., Rankin, C.C., Roybal, Y.R., Stelma, G.N., Scarpino, P.V. and Dupour, A.P. (1993). New medium for the simultaneous detection of total coliforms and *Escherichia coli* in water. *Appl. Environ. Microbiol.*, **59**, 3534–3544.

Burlingame, G.A., McElhaney, J., Bennett, M. and Pipes, W.O. (1984). Bacterial interference with coliform colony sheen production on membrane filters. *Appl. Environ. Microbiol.*, **47**, 56–60.

Cavari, B.Z., Allen, D.A. and Colwell, R.R. (1981). Effect of temperature on growth and activity of *Aeromonas spp*. and mixed bacterial populations in the Anacostia River. *Appl. Environ. Microbiol.*, **41**, 1052–1054.

Daubner, I. (1972). *Mikrobiologie des Wassers*. Akademie-Verlag, Berlin.

Edberg, S.C., Rice, E.W., Karlin, R.J. and Allen, M.J. (2000). *Escherichia coli*: the best biological drinking water indicator for public health protection. *J. Appl. Microbiol*, **88**, 106S–116S.

Franzblau, S.G., Hinnebusch, B.J., Kelley, L.M. and Sinclair, N.A. (1984). Effect of noncoliforms on coliform detection in potable groundwater: Improved recovery with an anaerobic membrane filter technique. *Appl. Environ. Microbiol.*, **48**, 142–148.

Gärtner, H. and Reploh, H. (1964). *Lehrbuch der Hygiene*. G. Fischer Verlag, Stuttgart.

Hazen, T.C., Fliermans, C.B., Hirsch, R.P. and Esch, G.W. (1978). Prevalence and distribution of *Aeromonas hydrophila* in the United States. *Appl. Environ. Microbiol.*, **36**, 731–738.

Leclerc, H., Mossel, D.A.A., Edberg, S.C. and Struijk, C.B. (2001). Advances in the bacteriology of the coliform group: Their suitability as markers of microbial water safety. *Annu. Rev. Microbiol.*, **55**, 201–234.

Kavka, G. (1978). Zur Frage der Nachweisbarkeit und Überlebenszeit von Kolibakterien in verschiedenen Biotopen. Thesis, Universitat Wien.

Kohl, W. (1975). Über die Bedeutung bakteriologischer Untersuchungen für die Beurteilung von Fließgewässern, dargestellt am Beispiel der österreichischen Donau. *Arch. Hydrobiol. Suppl.*, **44**, 392–461.

Lavoie, M.C. (1983). Identification of strains isolated as total and fecal coliforms and comparison of both groups as indicatiors of fecal pollution in tropical climates. *Can. J. Microbiol.*, **29**, 689–693.

Lee, Y.O. (1986). Beitrag zur Erfassung der jahreszeitlichen Veränderungen der Entero- bakteriaceen auf submersen Makrophyten und im freien Wasser. Thesis, Universitat Wien.

Lee, Y.O. (1996). Comparative studies on detection of *E. coli* in surface water. *Kor. J. Limnol.*, **29**, 313–321.