II-442 USE OF THE POLYMERASE CHAIN REACTION IN DETECTION OF GROUP III RNA-F-SPECIFIC COLIPHAGE IN RAW SEWAGE WASTEWATER AND NIGHT SOIL SAMPLES

S. Danteravanich and S. Ohgaki
Department of Urban Engineering, University of Tokyo.

1. Introduction

RNA-F-specific coliphages (FRNA coliphages) were demonstrated to have potential applications as indicator of sewage contamination, and efficiency of water and wastewater treatment. However, the ability to use FRNA coliphages as indicator of fecal contamination is still an open question. This was primarily due to the quantitative comparison of a high number of FRNA coliphages in sewage wastewater to a low number in human feces. The rare detection of FRNA coliphages from human feces could be explained either by the infrequent occurrence of these phages in feces or alternatively, by inadequate specific or sensitive detection method.

There are 4 groups of FRNA coliphages. Each group was significantly found to originate from different sources. The group III FRNA coliphage such as Qβ, ST, and VK phages was reasonably found generally in feces from human source (Furuse, K., 1987). The report described here, however, was primarily concerned with the use of polymerase chain reaction (PCR) technique as a specific detection method to determine the presence of group III FRNA coliphage in raw sewage and night soil samples. Raw sewage sample in which FRNA coliphages are detected will be further subjected to comfirmation to determine whether the contamination is from human feces or not.

2. Samples and Methods

The night soil and raw sewage wastewater samples were taken from Urayasu Sanitary Treatment Plant and Mikawashima Wastewater Treatment Plant, respectively. The FRNA coliphages concentration was determined from all samples. Some of the original samples were, at first, directly enriched to increase coliphages concentration, followed by destroying their capsids , and finally tested with PCR method. Separately , we randomly picked up 35-40 plaques from each original sample and single plaque isolation was done. The crude suspended isolated coliphage was screened to be FRNA coliphage by spot testing on E.coli K12, F', A/λ bacterial host with or without RNase A. The isolated phages that did not grow on bacteria host with the presence of RNase A were classifided as FRNA coliphages. These isolated FRNA coliphages were further identified to be group III FRNA coliphage by using PCR method.

The modified PCR method similar to that of described by Danteravanich, et al. (1992) which is based on utilizing Qβ coliphage as a defined model virus system was used to examine the group III FRNA coliphages in this study. However, the only difference in approach is that nested PCR was employed to increase the sensitivity of detection. The sensitivity of the PCR protocol employed in this investigation is illustrated in Table 1.

3. Results and Discussion

The sensitivity of PCR method is shown to be as little as 1 PFU of detectable

Qβ coliphage (Table 1), Based from this result, it seemed that PCR method has the ability to be an adequate, sensitive and specific method for detecting group III FRNA coliphage. The results of group III FRNA coliphage identification as shown in Fig. 1 and Table 2 show that this phage group was detected in both raw sewage wastewater and night soil samples. However, higher and more significant yield of group III FRNA phage was detected from raw sewage wastewater than night soil samples. This result is paralleled to the number of RNA-F-specific coliphages detected in raw sewage and night soil samples as summarized in Table 2.

Table 1. Sensitivity of PCR Protocol to Detect Qβ Coliphage.

PCR Technique	Primer Set	Detectable Qβ Coliphage (PFU)	
		1st PCR reaction	2nd PCR reaction
Dual PCR	1	8.1×10^{0}	8.1×10^{-4}
	2	8.1×10^{0}	8.1×10^{-1}
Nested PCR	1 & 2	8.1×10^{0}	8.1×10^{-4}

Note: - the 1st set of primer is composed of an antisense primer, 5'..ATTCACAATTAGGCGCCAT..3' and a sense primer, 5'..CCATCGATCAGCTTATCTGT..3' to amplify RNA fragment comprising 190 bp.
the 2nd set of primer is composed of an antisense primer, 5'..CTCGTGTAGAGACGCAACCTT..3' and a sense primer, 5'..GTCTACCGGCAAATTCGATAT..3' to amplify internal RNA fragment comprising 100 bp.

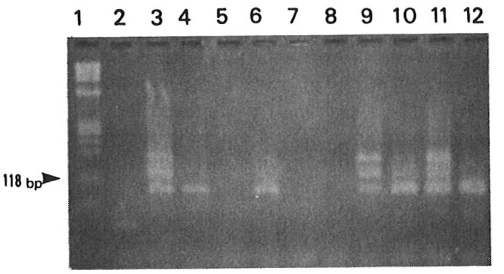

Fig. 2 Electrophoresis of nested PCR products for identification of group III FRNA coliphage in raw sewage wastewater and night soil samples. Lane 1: DNA marker; Lane 2 : negative control; Lane 3 and 9: positive control; Lane 4 - 8 : 1st-5th night soil samples; Lane 10: 5th raw sewage wastewater sample; Lane 11: 4th raw sewage sample; Lane 12: 3rd raw sewage sample.

Table. 2 RNA-F-specific coliphages concentration and identified group III FRNA coliphage in raw sewage wastewater and night soil samples.

Samples	Sampling Date	RNA-F-specific coliphages concentration (PFU/mL)	Number of plaque isolation	Positive plaque with RNase		Positive PCR Testing	
				number	percent	number	percent
NIGHT SOIL	27 Nov 1991	70	35	4	11.4	0	0
	17 Dec 1991	4	35	1	2.9	0	0
	31 Jan 1992	505	40	9	22.5	4	44
	7 Feb 1992	590	40	0	0.0	ND	ND
	17 Feb 1992	30	30	0	0.0	ND	ND
RAW SEWAGE	16 Oct 1991	2250	35	11	31.4	2	18
	3 Feb 1992	1300	40	1	2.5	0	0
	7 Feb 1992	1940	40	10	25.0	7	70
	10 Feb 1992	22700	40	27	67.5	26	96
	17 Feb 1992	2240	40	12	30.0	2	17

Note : ND = not done

Based from the results of the semi-quantitative detection of group III FRNA coliphage presented in this paper, the applicability of RNA-F-specific coliphages as indicator of fecal contamination is still inconclusive. The cause of the high number of FRNA coliphages in raw sewage is not understood. It could be due to either multiplication or introduce from other sources. Further investigation is needed to verify the proliferation of these phages in raw sewage.

References

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