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REVIEW

Development of the Multiplex PCR Detection Kit for *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7

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Abstract

This review describes the development of the multiplex PCR detection kit for *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in food samples. To develop a detection assay, our research team evaluated the optimization of the pre-enrichment broth, the simple DNA extraction method, and the multiplex PCR settings. When this detection protocol was used to detect the above pathogenic bacteria, one cell per 25 g of inoculated sample was detected within 24 h. Moreover, there was excellent agreement between the multiplex PCR assay and the conventional culture method. The multiplex PCR detection assay system was confirmed to be a reliable and useful method for the rapid screening of food products for foodborne pathogens. The assay system was commercialized as a "[TA10] Pathogenic Bacterial Multiplex PCR Detection Kit". When this kit was provided to four different laboratories for an extensive validation study, there were no significant differences in detection sensitivity among the laboratories. The detection kit will be valuable as a screening method for foods contaminated with these pathogens, and it will also be useful for identifying the sources of outbreaks of foodborne illness.

Discipline: Food
Additional key words: foodborne pathogens, rapid detection, rapid screening

Introduction

Foodborne illness caused by *Salmonella* spp., *Listeria monocytogenes*, or *Escherichia coli* O157:H7 is a major public health concern worldwide (McLauchlin et al., 1996[5]; Schlosser et al., 2000[7]; Wells et al., 2004[9]). There are approximately 1.4 million cases of illness annually, resulting in 1,000 deaths (Mead et al., 1999[6]; Buzby, 2000[1]). To prevent these outbreaks, the ability to rapidly detect these pathogens in food is critical.

Reliable detection techniques are a prerequisite for the detection and identification of these pathogenic bacteria in foods, food sources, and food processing plants. Because the conventional culture method for detecting pathogens (Fig. 1., Left side) is time consuming, results are frequently not available until the food has been either released to the market or consumed, thus increasing the risk of transmission of pathogens. Pathogens are often present in very low numbers against a background of indigenous microflora, rendering the recovery of target organisms difficult. Rapid and sensitive assays with high specificity are required for the detection of pathogenic bacteria in foods and other types of samples. PCR-based methods have the potential for the rapid and sensitive detection of foodborne pathogens. Because PCR can target unique genetic sequences, such as the virulence genes of microorganisms, it has the advantage of being an extremely specific assay.

The multiplex PCR method is capable of determining the presence of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 directly from enrichment cultures by targeting the specific DNA sequences of each pathogen (Kawasaki et

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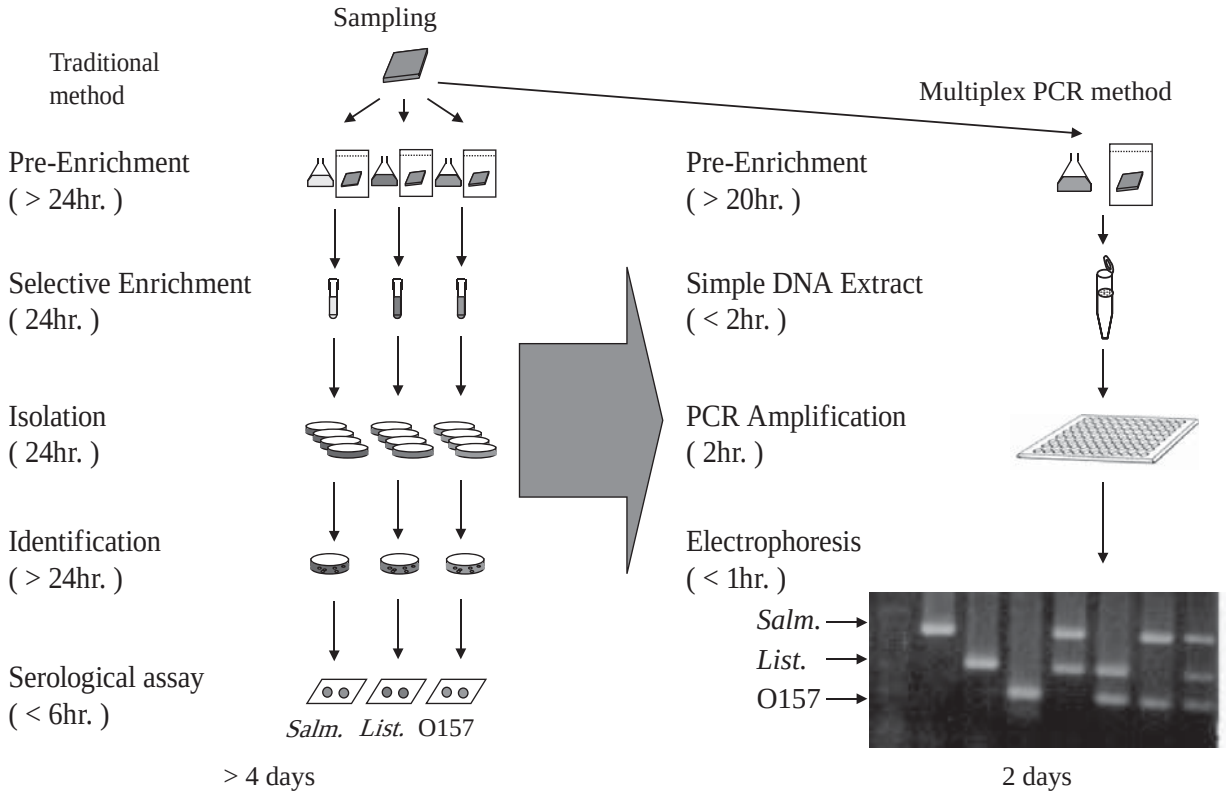

Fig. 1. Schematic representation of detection procedure of conventional culture method and multiplex PCR method

al., 2005[3]). This multiplex PCR method was used to detect pathogens in spiked pork samples, and the detection sensitivity for each pathogen was 1 CFU per 25 g food sample after enrichment for 24 hours. Moreover, there was excellent agreement between the results of the multiplex PCR and the conventional culture method in naturally contaminated meat samples.

This review describes the multiplex PCR method for screening various types of food samples for pathogens and the development of a detection kit for foodborne pathogenic bacteria.

Overview of the multiplex PCR detection protocol

The schematic representation of the conventional culture method and the multiplex PCR detection method is shown in Figure 1. This multiplex PCR method is simple to use, and the results are typically available within 24 h compared to at least four days for the conventional culture method. The multiplex PCR detection protocol consists of three steps: 1) pre-enrichment culturing, 2) simple DNA extraction, and 3) multiplex PCR detection. Firstly, an enrichment medium allows the simultaneous growth of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 for subsequent detection of each pathogen using a multiplex PCR assay with similar sensitivity. Secondly, a simple DNA extraction method without phenol and chloroform ensures not only the high sensitivity of the multiplex PCR assay but also safety in handling for practical use. Finally, the multiplex PCR component concentrations have been optimized for the specific detection of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7. The detection limit of individual target strains for multiplex PCR is shown in Fig. 2. The correct PCR product was clearly detected in each of the target genomic DNA samples estimated to contain 1 cell/PCR (10^3 CFU/ml of culture) (Kawasaki et al., 2005[3]).

Application to various food matrices

This multiplex PCR detection method has high sensitivity, since one cell per PCR reaction tube was detectable. Therefore, the multiplex PCR is a useful method for the rapid screening contaminated food for *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7. There have been many reports on pathogen detection using PCR methods for various foods, such as chicken, milk, ground beef, etc. (Thomas et al., 1991[8]; Croci et al., 2004[2]). However, many of these reports described detection from pure cultures or from a specific food matrix. There have been few reports describing sample treatments conducted prior to PCR to remove PCR inhibitors from a variety of food matrices followed by the detection of pathogenic bacteria.

To evaluate the practical use of the multiplex PCR

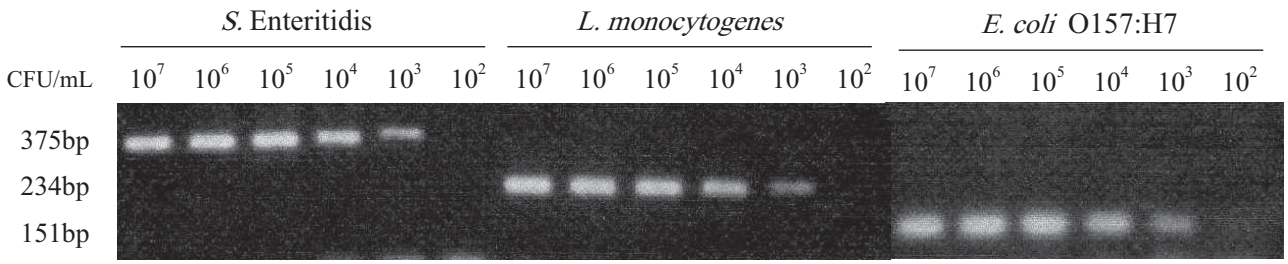

Fig. 2. The detection limit of individual target strains for the multiplex PCR method

Table 1. Results obtained with the multiplex PCR and conventional culture methods from the retail food samples

Sample	*Salmonella* spp.		*Listeria monocytogenes*		*E. coli* O157:H7	
	PCR	Conventional	PCR	Conventional	PCR	Conventional
Chicken	12 / 37	9 / 37	11 / 37	11 / 37	0 / 37	0 / 37
Pork	1 / 14	0 / 14	1 / 14	2 / 14	1* / 14	0 / 14
Beef	0 / 7	0 / 7	2 / 7	1 / 7	0 / 7	0 / 7
Minced meat (Pork and Beef)	0 / 3	0 / 3	1 / 3	0 / 3	0 / 3	0 / 3
Sea food	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5
Others	0 / 9	0 / 9	0 / 9	0 / 9	0 / 9	0 / 9
Total	13 / 75	9 / 75	15 / 75	14 / 75	1 / 75	0 / 75

* : O55 was detected from culture method

Table 2. Results obtained with the multiplex PCR and conventional culture methods in the frozen food samples after storage at –20°C

Storage time	Positive sample number					
	Salmonella Enteritidis		*Listeria monocytogenes*		*E. coli* O157:H7	
	PCR	Conventional	PCR	Conventional	PCR	Conventional
2 weeks	27 / 28	13 / 28	27 / 28	26 / 28	26 / 28	20 / 28
2 months	24 / 28	6 / 28	24 / 28	22 / 28	23 / 28	18 / 28

method for detecting the three pathogens in foods, the conventional culture method was compared to the PCR assay using 75 commercial food samples (Table 1). For *E. coli* O157:H7, one sample (pork intestine) was found positive using the multiplex PCR method, but this *E. coli* isolate was confirmed to be *E. coli* O55 by the conventional culture method and serological testing. Of the 75 food samples, 13 were found positive for *Salmonella* spp. by the multiplex PCR method, but only nine samples were positive by the conventional culture method. For *L. monocytogenes*, 15 samples were positive by the multiplex PCR assay compared to 14 samples by the conventional culture method.

The multiplex PCR assay was also performed on spiked frozen food samples. The detection frequency of the pathogens from 28 samples of meat, cabbage, salmon, raw egg, milk, fresh cheese, and raw ham stored at -20°C for periods of two weeks and two months is shown in Table 2. The detection rate for each pathogen using multiplex PCR was higher than that of the conventional culture method in all the post-storage frozen samples. The detection rate decreased using both methods on samples stored for two months at -20°C compared to those stored for two weeks. This was evident particularly for *Salmonella* Enteritidis since detection by the culture method declined considerably after frozen storage. The detection rate for each pathogen by multiplex PCR was greater than 75% for all food samples after frozen storage for two months (Kawasaki et al., 2009[4]).

Benefits to food safety, the laboratory worker, and the food industry

The development of a multiplex PCR method and the evaluation of detection sensitivity for *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 in various spiked frozen and non-frozen food samples and with various naturally contaminated foods prompted us to develop a multiplex PCR detection kit for quality control in the food industry.

However, an extensive validation study was performed to determine the reproducibility of the detection kit. Initially, the multiplex PCR detection kit was prepared and provided to four domestic food companies for detecting the pathogens in food products in a collaborative study. The enrichment medium, DNA extraction kit, and multiplex PCR kit were made available and supplied by a Japanese

commercial company (Fig. 3). This detection kit "[TA10] Pathogenic Bacterial Multiplex PCR Detection Kit (URL : http://www.primaham.co.jp/kiso/hukusu.html)" was used for the validation study at the food companies.

A total of 55 types (cheese, apple juice, meat, fresh vegetable, fruits, etc.) of spiked food samples (1 CFU per 25 g inoculation level) were evaluated with the detection kits, and the results were compared with the conventional culture method in each collaborative laboratory. The enrichment broth [TA10] allowed detection of pathogenic bacteria below the lowest PCR detection limit (10^3 CFU/ml) within 20 h of incubation at 35°C, and the DNA extraction kit [TA10] was useful for preparing template DNA from various food samples. A detection limit of 1 CFU per 25g for each pathogen was observed with the commercial detection kit. A comparison of results from the multiplex PCR and the conventional culture method is presented in Fig. 4.

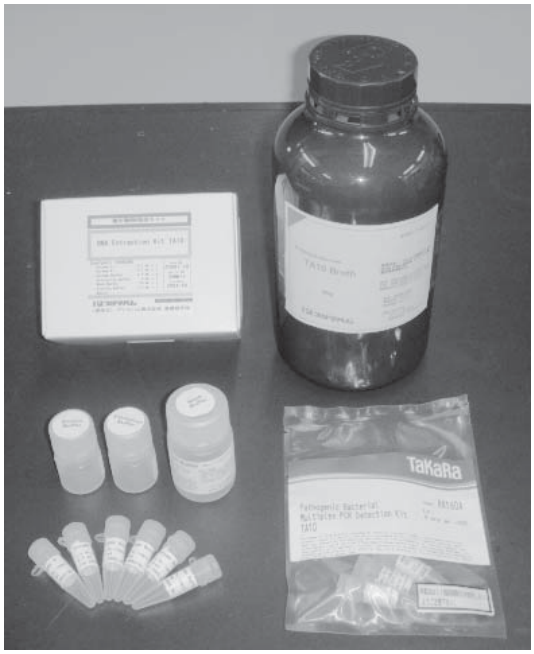

Fig. 3. Pathogenic bacterial multiplex PCR detection kit [TA10]

The [TA10] Pathogenic Bacterial Multiplex PCR Detection Kit (including pre-enrichment broth, DNA extraction kit, and multiplex PCR kit) can simplify food testing and provide higher detection sensitivity for various food samples. There were no significant differences in the detection sensitivity of the [TA10] PCR detection kit among the companies that tested it. Therefore, this commercial kit provides a useful tool to ensure the safety of food tested by inspection laboratories.

Conclusion

The multiplex PCR detection kit is useful to industries and consumers for enhancing food safety. However, an extensive validation study needs to be performed by a third party both nationally and internationally to reproduce the data and to make the technology available to the food industry. Any initiative to improve this technology by a third party will be much appreciated.

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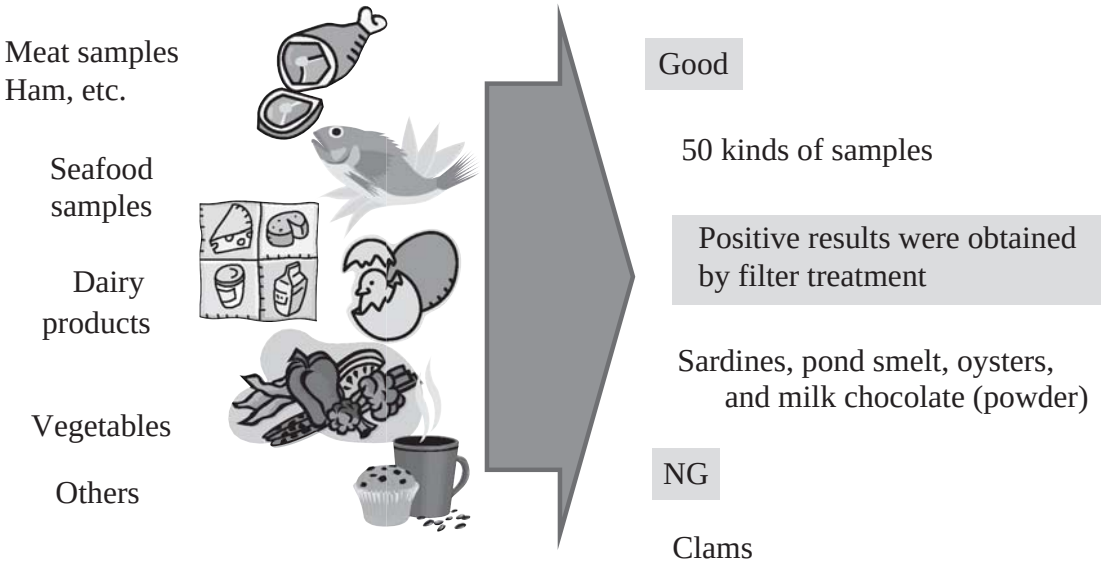

Fig. 4. Evaluation of multiplex PCR Kit in various food matrices

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