SIMULTANEOUS DETECTION OF FIVE FOOD-BORNE PATHOGENS USING CAPILLARY ELECTROPHORESIS-BASED SINGLE-STRAND CONFORMATION POLYMORPHISM IN MINCED MEAT

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***Abstract* - Capillary electrophoresis-based single-strand conformation polymorphism (CE-SSCP) coupled with multiplex PCR method was developed for the detection of five pathogens associated with food poisoning, including *Salmonella enterica* Typhimurium, *Listeria monocytogenes, Shigella sonnei, Yersinia enterocolitica,* and *Escherichia coli* O157:H7. The method was applied to model food systems, both of culture medium and minced meat, and the detection limit of the mixture of five microbes was 10^3 CFU/g.**

***Index Terms* - food-borne pathogens, CE-SSCP, multiplex PCR**

I. INTRODUCTION

Increased public awareness of the health-related and economic impact of food-borne contamination and illness has resulted in greater efforts to develop more rapid and sensitive methods of identifying and detecting pathogens. Capillary electrophoresis (CE) is a relatively new separation technology that uses a narrow capillary format and has multiple benefits for analysis of molecules that are of clinical interest. It has been used for sequencing (Carriho, 2000), fragment size analysis (Butler, Wilson & Reeder, 1998), and mutation-detection analysis (Andersen, Jespersgaard, Vuust, Christiansen & Larsen, 2003). In recent years, CE combined with single strand conformation polymorphism (SSCP) has also been used in the diagnosis of pathogens (Larsen, Jespersgaard & Andersen, 2007; Oh, Park, Paek, Kim, Jung & Oh, 2008; Shin, Cho, Hwang, Park & Jung, 2008; Zinger et al, 2007). In this study, we developed CE-SSCP based on multiplex PCR amplification for the simultaneous detection of five food-borne pathogens, including *Salmonella enterica* Typhimurium, *Listeria monocytogenes, Shigella sonnei*, *Yersinia enterocolitica,* and *Escherichia coli* O157:H7, in the minced meat samples.

II. MATERIALS AND METHODS

Bacterial strains and preparation of minced meat sample

The reference strains used are *Salmonella enterica* Typhimurium ATCC 13311 & ATCC 19585, *Listeria monocytogenes* ATCC 15313 & ATCC 19111, *Shigella sonnei* KCTC 2581, *Yersinia enterocolitica* ATCC 23715, *Escherichia coli* ATCC 27325 and *Escherichia coli* O157:H7 ATCC 43894.

Minced meat (10 g) was transferred into a sterile stomacher bag containing a filter and inoculated with bacterial species to obtain an approximate cell concentration at 10^3 CFU/g. Inoculated sample was mixed with 90 mL BPW and homogenized using a Stomacher for 2 min. One milliliter of this mixture was subjected to DNA extraction using the DNeasy kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instructions.

Multiplex PCR and CE-SSCP conditions

PCR amplification of DNA from samples was performed using the AccuPower® *HF* PCR premix (Bioneer Inc., Daejeon, Korea). All reverse primers were labeled at the 5′ end with a hexachloro derivative of fluorescein (HEX). The PCR reaction parameters were 5 min at 95°C, followed by 30 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec at 55°C and extension for 35 sec at 72°C, and a final extension at 72°C for 7 min.

CE-SSCP analysis was performed as previously described (Oh et al., 2008). Briefly, 1 μL of sample was mixed with 13.5 μL of deionized formamide (Applied Biosystems, Inc.) and 0.5 μL of ROX 500 size standard (Applied Biosystems, Inc.) to measure the position of the peak. The samples were denatured at 95°C for 4 min. CE-SSCP analysis was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Inc.), which was set up according to the manufacturer's instructions. The signal was automatically analyzed, and the positions of the peaks were determined using DNA analysis software (GeneMapper, Applied Biosystems, Inc.).

III. RESULTS AND DISCUSSION

Five foodborne bacteria, including *Salmonella enterica* Typhimurium, *Listeria monocytogenes*, *Shigella sonnei*, *Yersinia enterocolitica*, and *Escherichia coli* O157:H7 were chosen as a model set for development of a simultaneous detection method using CE-SSCP. The specific primers used in this study and data points corresponding to individual pathogens shown in Table 1. All peaks were separate and distinctly positioned within the 1980 to 3300 range of normalized data points; none of the reactions generated more than a single peak. After verification of the separate individual electropherograms, a mixture of the five pathogens was subjected to CE-SSCP combined with multiplex PCR. The peaks in the sample containing all five target pathogens were located at the same positions as those obtained from the PCR products from the individual sample (data not shown). The detection of the CE-SSCP method was also determined for each pathogen species in model and real food systems. Single pathogens from both pure cultures and artificially inoculated minced meat samples were detected in concentrations at 10^3 CFU/mL. Based on this result, a mixture of five pathogens was prepared containing 10^3 CFU/mL of each species and analyzed by the CE-SSCP method. As shown in Fig. 1, all five pathogens were detected in minced meat, and exhibited the same peak position as results from individual samples with SSCP electrophoresis.

IV. CONCLUSION

The CE-SSCP based on multiplex PCR assay method developed could directly detect as low as 10^3 CFU/mL of five pathogens in a single run without any additional steps. As the developed method can be performed within 6 hr from sampling to obtain final results, our approach presented here offers a sensitive, rapid and simple method to detect and identify food-borne pathogens in minced meat sample.

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Table 1. Primers used and data point of individual pathogens in CE-SSCP

Bacterial strain	Primers (5'-3')	Product size (bp)	Reference	Data point
S. enterica Typhimurium	CGGGGAGGAAGGTGTTGTGG GCCAGCAGCCGCGGTAA	91	Oh et al (2008)	1984.00
E. coli 0157:H7	CAGGTGAAGGTGGAATGGTTGTC TTAGAATTGAGACCATCCAATAAG	296	Bertrand et al (2007)	2688.37
L. monocytogenes	GCGCATGCCACGCTTTTG GCCAGCAGCCGCGGTAA	351	Oh et al (2008)	2788.49
Y. enterocolitica	GTAGTTTACTACTTTGCCGG GCCAGCAGCCGCGGTAA	455	Oh et al (2008)	3017.47
S. sonnei	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGAGTAC	600	Kim et al (2006)	3292.00

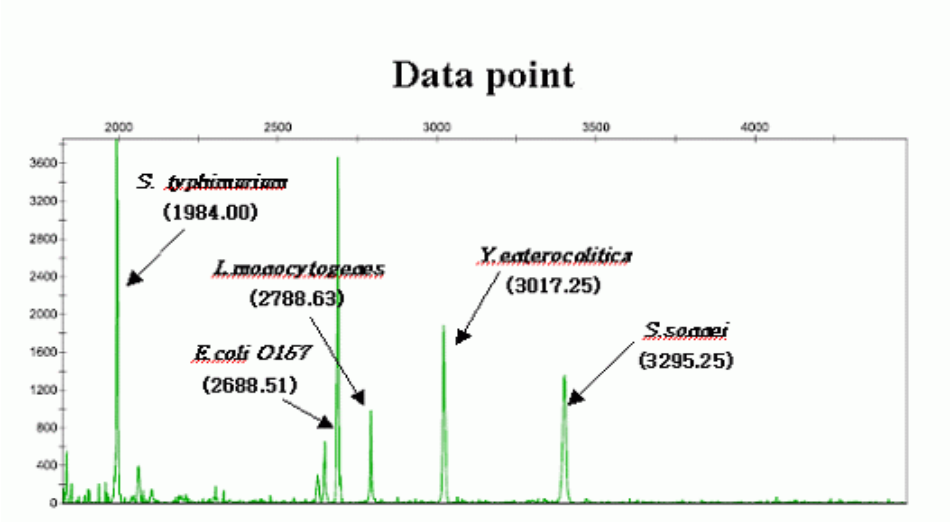

Fig. 1. Electropherogram of multiplex PCR results in minced meat sample.