A Multiplex PCR Assay for the Detection of Food-borne Pathogens in Meat Products

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Abstract
Meat and meat products are a potential source of food-borne pathogens, including Staphylococcus aureus, Salmonella spp., Escherichia coli O157:H7, and Bacillus cereus. A sensitive and specific PCR assay for the detection of these pathogens in meat and meat products was developed in this study, as part of a broader effort to reduce the potential health hazards posed by these pathogens. Initially, PCR conditions were standardized with purified DNA. Under standard conditions, the detection level for PCR was as low as 10 pg of purified bacterial DNA. After overnight growth of bacteria in a broth medium, as few as 10^2 CFU of bacteria were detected by PCR assay. The primers employed in the PCR assay were found to be highly specific for individual organisms, and evidenced no cross-reactivity with heterologous organisms. Additionally, the multiplex PCR assays also amplified some target genes from the four pathogens, and multiplex amplification was obtained from as little as 10 pg of DNA, thus illustrating the excellent specificity and high sensitivity of the assay. In conclusion, this PCR-based technique provides a sensitive and specific method for the detection of S. aureus, Salmonella spp., E. coli O157:H7, and B. cereus in meat and meat products.

Key words: meat product, food safety, food-borne pathogen, multiplex PCR assay

Introduction
The microbiological safety of food constitutes a significant concern for both consumers and industries today. The rapid and accurate identification of bacterial pathogens in foods is important with regard to both quality assurance and the tracing of bacterial pathogens within the food supply (Bhagwat, 2003). In 2000, 49% of patients visiting hospitals for the treatment of food-borne diseases had consumed contaminated meat or meat products, and the incidence of food-borne diseases caused by pathogenic bacteria is increasing annually in Korea (Kim et al., 2008).

Escherichia coli O157:H7 is an important verotoxin-producing strain of E. coli (VTEC) that is generally associated with food-borne and water-borne infections (Sarimehmetoglu et al., 2009). The pathogen has been implicated in large food-borne outbreaks all over the world, including in Canada, UK, Scotland, and Japan. Escherichia coli O157:H7 has been shown to cause several disease syndromes, including diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura in humans (Choi et al., 2010; Gooding and Choudary, 1997). Additionally, enterotoxigenic Staphylococcus aureus is one of the major pathogens responsible for cases of food poisoning all over the world (Dinges et al., 2000). The emetic staphylococcal enterotoxins (SE) are classified as members of the pyrogenic toxin superantigen family, owing to their biological activities and structural relatedness (Balaban and Rasooly, 2000; Dinges et al., 2000). Eleven major antigenic types of SEs have been recognized thus far (SEA to SEJ) (Jarraud et al., 1999; Monday and Bohach, 1999; Tamarapu et al., 2001), and their corresponding genes have been reported in previous studies (Munson et al., 1998). Strains of Salmonella can cause general infection, food poisoning, and salmonellosis, a zoonotic disease of significant importance (Davies and Hinton, 2000). Salmonellosis and listeriosis are two of the most common food-borne diseases (Anonymous, 2001; Mead et al., 1999), and early and sensitive detection is a critical issue in pub-
lic health policy (Rijpens and Herman, 2002). *Bacillus cereus* is a Gram-positive, spore-forming, facultative-aerobic bacteria. *B. cereus* is the dominant aerobic bacterium in cooked, pasteurized, and chilled products, owing to the ability of spores to survive during the pasteurization process. *Bacillus* spp. have also been detected in other cooked and chilled foods (Choma *et al*., 2000; Simpson *et al*., 1994).

The development of rapid, sensitive, and infallible methods for the detection of food-borne pathogens has received a great deal of attention in recent years, owing to an increased public awareness regarding the health hazards associated with microbial contamination of food. Current methods for the detection of food-borne pathogens generally involve the following: (a) colony isolation on selective media, (b) the use of biochemical tests, and (c) serotyping using antibodies against specific bacterial antigens. These procedures are both cumbersome and time-consuming. In certain cases, it can take several days to establish the identity of particular bacteria. Therefore, new approaches to milk safety are necessary for the rapid and efficient detection of the low numbers of bacteria likely to be present in milk. In recent years, several methods have been tested to facilitate the identification of bacteria in foods. Molecular techniques such as PCR have been extensively employed for several years for the identification and characterization of bacteria in food samples, including meat and dairy products (Hill, 1996; Wang *et al*., 1997). However, these assays involved the use of selective enrichment techniques to recover bacteria in food samples, and 48-72 h were required for confident identification of the bacteria. Conventional methods of bacteria detection in food involve propagation in selective enrichment media, followed by microbiological and biochemical tests, which are both cumbersome and time-intensive. The advent of nucleic-acid-based assay systems such as the polymerase chain reaction (PCR) has resulted in the emergence of improved, expedient, and reliable microbial identification and surveillance techniques, which are capable of detecting even nonviable cells (Josephson *et al*., 1993; Scheu *et al*., 1998). The direct detection of pathogenic bacteria in food samples constitutes a challenging task, hampered by the presence of PCR-inhibitory substances frequently associated with enrichment media, DNA isolation reagents and the food matrix itself, and further compounded by the presence of large numbers of indigenous microflora (Rossen *et al*., 1992; Wilson, 1997). Consequently, a clear need exists for the development of a sample preparation strategy that can effectively sequester the pathogenic bacteria or the target DNA from the food sample. In order to achieve this improved sample preparation, methods have been designed on the basis of enzymatic treatment, buoyant density centrifugation, DNA affinity purification columns, and magnetic beads coated with specific antibodies or lectins (Lindqvist *et al*., 1997; Par-Gunnar *et al*., 1994; Powell *et al*., 1994).

Meat and meat products are a potential source of food-borne pathogens, including *S. aureus, Salmonella* spp., *E. coli* O157:H7, and *B. cereus*. According to the Korean Ministry for Food, Agriculture, Forestry and Fisheries (2007), the revised Processing Standard and Ingredients Standard of Livestock Product classifies meat products as “processed meat products”, and thus the levels of pathogenic bacteria (i.e. *S. aureus, Salmonella* spp., *E. coli* O157:H7 etc.) do not need to be assessed, according to current regulations. A more sensitive and specific method for the detection of these pathogens in meat and meat products will help to reduce the potential health hazards they pose.

The principal objective of this study was to evaluate the reliability and applicability of using a multiplex PCR assay with built-in quality assurance measures that can be employed after culture enrichment for the routine examination of meat products for *S. aureus, Salmonella* spp., *E. coli* O157:H7, and *B. cereus*.

**Materials and Methods**

**Bacterial strains and DNA extraction**

The bacterial strains employed herein are listed in Table 1. The bacterial species and their origins are as follows: *Staphylococcus aureus* ATCC 25923, *Salmonella Enteritidis* KCCM 12021, *Escherichia coli* O157:H7, and *Bacillus cereus* KCCM 11341. *S. aureus* ATCC 25923, and *E. coli* O157:H7 were obtained from the National Veterinary Research and Quarantine Service (NVRQS, Anyang, Korea), and *S. Enteritidis* KCCM 12021 and *B. cereus* KCCM 11341 were acquired from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). Cultures of *S. aureus* ATCC 25923, *S. Typhimurium* KCCM 12021, *E. coli* O157:H7, and *B. cereus* KCCM 11341 were initiated from freezer stocks and grown in Tryptic Soy Agar (Difco Laboratories, MD, USA). After overnight incubation at 37°C, a single colony was selected and inoculated into 50 mL of Tryptic Soy Broth in a 250 mL Erlenmeyer flask. The cells were grown for 20-24 h at 37°C with shaking at 200 rpm.

Genomic DNA was isolated with an AccuPrep® genomic