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Recent Advance on the Methods for Rapid Detection of Foodborne Pathogens

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ABSTRACT—*Rapid detection and identification of foodborne pathogens is an important prerequisite for timely and effective control and prevention of pathogen spread and food poisoning. Culture-based methods are too time-consuming for high-throughput testing and too tedious for analysis of samples with multiple organisms and provide little clinical information regarding the pathogen. With continued development of novel molecular-based technologies for rapid, high-throughput detection of foodborne pathogenic bacteria, a number of unique approaches and variations on existing techniques are currently on the market or are being implemented that offer ease of use, reliability and low cost. In this paper, the most frequently used assays for foodborne pathogens, including immunomagnetic separation, DNA-hybridization, capillary zone electromigration, conventional PCR, multiplex PCR, real-time PCR, DNA microarray, LAMP and biosensors were reviewed.*

Keywords—foodorne pathogen, detection, methods, food safety

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1. INTRODUCTION

Food, the material basis of human survival, is directly related to human health. In recent years, food safety has been attached great importance all over the world and has become a significant concern due to increasing numbers of outbreaks and the resulting fatalities and economic losses. Foodborne disease caused by pathogenic microorganisms is one of the most important factors to food safety. Therefore, rapid detection and identification of food pathogens is an important prerequisite for timely and effective control and prevention of pathogen spread and food poisoning.

Culture-based methods used for food pathogens detection and identification are simple to use, relatively inexpensive, and sensitive. However, culture-based methods are too time-consuming for high-throughput testing and too tedious for analysis of samples with multiple organisms and provide little clinical information regarding the pathogen (Rasooly and Herold, 2008). With continued development of novel molecular-based technologies for rapid, high-throughput detection of foodborne pathogenic bacteria, the future of conventional microbiological methods seems tenuous (Gracias and McKillip, 2004). This paper reviewed the most frequently used assays for foodborne pathogens, including immunomagnetic separation, DNA-hybridization, capillary zone electromigration, conventional PCR, multiplex PCR, real-time PCR, DNA microarray, LAMP and biosensors.

2. IMMUNOMAGNETIC SEPARATION

Salmonella could be separated from different inoculated foods using antibody-coated immunomagnetic beads. When applied on suitable foods, the immunomagnetic separation technique showed a sensitivity of 10–20 *Salmonella* cells/g of the original sample (Skjerve and Olsvik, 1991). Rijpens et al. (1999) described the rapid detection of an average of 5.9 stressed *Salmonella* cells in 25 g of food product using immunomagnetic separation in dairy and egg products. Fu et al. (2005) developed a method combining immunomagnetic separation and real-time PCR to detect *Escherichia coli* O157:H7. The minimum detection limit was $<5\times10^2$ cells/ml for suspensions of *E.coli* O157:H7 in buffer and 1.3×10^4 cells/g for *E.coli* O157:H7 in ground beef.

3. CAPILLARY ZONE ELECTROMIGRATION

The main title (on the first page) should be centered, and in Times New Roman 18-point, boldface type. Capitalize the first letter of nouns, pronouns, verbs, adjectives, and adverbs; do not capitalize articles, coordinate conjunctions, or prepositions (unless the title begins with such a word). Please initially capitalize only the first word in (for example, "Format for Preparation of Paper for Publication in the AJCIS" — as in these guidelines). Capillary zone electrophoresis was found to be popular for the analyses of food pathogens. The analysis of food components including amino acids, biogenic amines, peptides, proteins, DNAs, carbohydrates, phenols, polyphenols, pigments, toxins, pesticides, vitamins, additives, small organic and inorganic ions and other compounds based on capillary electromigration techniques were reviewed by García-Cañas and Cifuentes (Electrophoresis, 2014, 35, 147-169), Herrero et al. (Electrophoresis, 2010, 31, 205-228), Castro-Puyana et al. (Electrophoresis, 2012, 31, 147-167) and Rabanes et al. (Electrophoresis, 2012, 31, 180-195).

The simultaneous detection of *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* spp. has been approached by multiplex PCR-based procedure followed by capillary gel electrophoresis with laser-induced fluorescence detection (multiplex-PCR-CGE-LIF) (Alarcón et al., 2004). As compared to slab gel electrophoresis, the use of CGE-LIF improved from 10- to 1000-fold the sensitivity of the multiplex PCR analysis, allowing the detection of 2.6×10^3 cfu/ml of *S.aureus*, 570 cfu/ml of *L.monocytogenes*, and 790 cfu/ml of *Salmonella* in artificially inoculated food, without enrichment. Following 6 h of enrichment, as low as 260, 79, and 57 cfu/ml of *S.aureus*, *L.monocytogenes* and *Salmonella*, respectively, were detected. The CGE-LIF method is shown to be reproducible, providing relative standard deviation values lower than 0.8% for analysis time and lower than 5.8% for peak areas.

4. MOLECULAR-BASED TECHNOLOGIES

With continued development of novel molecular-based technologies for rapid, high-throughput detection of foodborne pathogenic bacteria, a number of unique approaches and variations on existing techniques are currently on the market or are being implemented that offer ease of use, reliability and low cost.

4.1 DNA-hybridization

16S rRNA-targeted oligonucleotide probes were used in a whole cell *in situ* hybridization assay for the identification of the genus *Leuconostoc* and an undescribed *Leuconostoc* ribospecies (Olsen et al. 2007). The probes were fluorescently labelled and used to quantify the *Leuconostoc* populations in five different mixed starter cultures.

The DNA Checkerboard method enables the simultaneous identification of distinct microorganisms in a large number of samples and employs up to 45 whole genomic DNA probes to gram-negative and gram-positive bacterial species present in subgingival biofilms. Similarly do Nascimento et al. (2009) presented the DNA checkerboard hybridization as an alternative method for the detection and quantitation of Candida species in oral cavities.

4.2 DNA microarray

Microarray analysis is an emerging technology that has the potential to become a leading trend in bacterial identification in food and feed improvement (Al-Khaldi et al. 2002). Oligonucleotide microarrays are widely used for analysis of gene expression, but the same technology can also be used for genotyping and for analysis of microbial pathogens for food safety, medical, and environmental applications (Sergeev et al. 2006). In combination with bioinformatics, DNA microarray technology provides unparalleled opportunities for simultaneous detection of thousands of genes or target DNA sequences and offer tremendous potential for studying foodborne microorganisms (Kostrzynska and Bachand, 2006). Donhauser et al. (2011) reported a chemiluminescence flow-through DNA microarray assay for the rapid and sensitive quantification of the pathogenic bacteria *E.coli* O157:H7, *S.enterica*, and *C.jejuni* in water. Wang et al. (2007) described a rapid (<4 h) high-throughput detection and identification system that uses universal polymerase chain reaction (PCR) primers to amplify a variable region of bacterial 16S rRNA gene, followed by reverse hybridization of the products to species-specific oligonucleotide probes on a chip. This procedure was successful in discriminating 204 strains from pure culture belonging to 13 genera. When this method was applied directly to 115 strains of bacteria isolated from foods, 112/115 (97.4%) were correctly identified; two strains were indistinguishable due to weak signal, while one failed to produce a PCR product. The array was used to detect and successfully identify two strains of bacteria from food poisoning outbreak samples, giving results through hybridization that were identical to those obtained by traditional methods. The sensitivity of the microarray assay was 10^2 cfu of bacteria. Wang et al. (2009) developed a rapid, DNA microarray-based detection technique to identify 10 different pathogenic bacteria associated with powdered infant formula (PIF) contamination based on the 16S-23S rRNA gene internal transcribed spacer (ITS) sequences and *wzy* (O antigen polymerase) gene. Using this procedure, *E.sakazakii*, *S.enterica*, *K.pneumoniae*, *K.oxytoca*, *S.marcescens*, *A.baumannii*, *B.cereus*, *L.monocytogenes*, *S.aureus*, and

E.coli O157 were successfully identified. Gardner et al. (2010) designed a pan-microbial detection array to detect all known viruses (including phages), bacteria and plasmids and developed a novel statistical analysis method to identify mixtures of organisms from complex samples hybridized to the array.

4.3 PCR

PCR can be used to amplify genes specific to taxonomic groups of bacteria and also to detect genes involved in the virulence of foodborne bacteria and shortens conventional microbiological methods for the detection of food pathogens either by replacing the conventional biochemical and serological identification or by its direct use on pre-enrichment media or food products (Rijpens and Herman, 2002). PCR method allows fast and highly reliable identification of bacterial taxa, particularly phenotypically atypical bacterial strains. PCR methods have been developed as an effective, rapid, reliable and sensitive technique for the detection of *fimA* gene of *Salmonella* (Hill, 1996). Riyaz-Ul-Hassan et al. (2004) indicated that the *Salmonella* enterotoxin (*stn*) gene was highly conserved and could be used as rapid and reliable method for detection of *Salmonella* spp. in water, milk and blood samples by PCR method with detecting less than 10 cells of *Salmonella* in 250 µl of blood and approx. 1 cell in 1 ml of water without any enrichment. Two pair of primers based on specific gene *fimA* of *Salmonella* and gene *afa* of pathogenic *E. coli* were designed for detecting *Salmonella* and pathogenic *E. coli*, respectively (Naravaneni and Kaiser, 2005). Trevanich et al. (2010) applied an optimized 18-h method involving one step culturing and single primer-based PCR assay for detection of *Salmonella* spp. in foods.

A nested multiplex PCR (nm-PCR) assay was developed to detect the presence of *Salmonella* in estuarine water and sediment samples (Touron et al. 2005). The target gene used was the phase 1 flagellin *fliC* chromosomal gene, present in all *Salmonella* serovars. A set of 4 primers was first used to amplify an 890-bp sequence of the *fliC* gene, and then a second set of 3 primers was used for the nested PCR. The nmPCR method has been successfully tested for 28 serovars, 13 of which are of epidemiological significance. The detection limit of the assay, without any pre-enrichment step, was estimated at 1 cfu in deionized water, and at 4-5 cfu in the reaction mixture when tested on estuarine water seeded with a *Salmonella* strain. When the nmPCR was used together with the classical culture method in environmental samples, it gave additional positive results for 11.3% of the sediment samples and 20% of the water samples despite a high background of other bacteria. Specific primers for multiplex PCR amplification of the shiga-like toxin (verotoxin type II), *femA* (cytoplasmic protein), *toxR* (transmembrane DNA binding protein), *iap* (invasive associative protein), and *invA* (invasion protein A) genes were designed to allow simultaneous detection of *E.coli* O157:H7, S.aureus, *V.*parahaemolyticus, *L.*monocytogenes, and *Salmonella*, respectively (Kim et al. 2007). Gordillo et al. (2011) developed procedure based on *fliC* and *rfbE* genes was for the detection of *E.coli* O157:H7 in raw pork meat and ready-to-eat meat products. The optimized PCR included an enrichment step in brilliant green bile 2% broth at 37℃. This method was applied to artificially inoculated meat and ready-to-eat meat products with different concentrations of *E. coli* O157:H7. Park et al. (2011) reported two types of PCR assays that could detect and quantify three pathogens, *Campylobacter* spp., *E. coli* O157:H7, and *Salmonella* spp., in watershed samples. Under optimized m-PCR conditions, the assay produced a 90-bp product for *C.jejuni*, a 150-bp product for *E.coli* O157:H7, and a 262-bp product for *S.*Typhimurium, and the limit of detection was approximately 700 copies.

Spano et al. (2005) performed real-time PCR for the detection of *Escherichia coli* O157:H7 in dairy and cattle wastewater with 10^4, 10^7 and 10^8 cfu/ml of *E.coli* O157:H7 strain EDL 933. The sensitivity limit of the assay was 10^{-1} pg/microl for eae, stx2 and 16SrRNA, and 1pg/microl for stx1 gene respectively. O' Grady et al. (2008) designed a novel diagnostic target (*ssrA* gene, 162bp) to detect *L.monocytogenes* in enriched food samples based real-time PCR. Application of the assay in natural and artificially contaminated culture enriched foods, including soft cheese, meat, milk, vegetables and fish, enabled detection of 1-5 cfu *L.monocytogenes* per 25 g/ml of food sample in 30 h. The specificity of the multiplex real-time PCR assay, using 128 pure-cultured bacteria including 110 *Salmonella* isolates and 18 non-*Salmonella* isolates, was 100%, 100% and 99.1% for *Salmonella* spp., *S.*Typhimurium and *S.* Enteritidis, respectively. The sensitivity was 100%, 100% and 91.7% for *Salmonella* spp., *S.*Typhimurium and *S.*Enteritidis, respectively. The multiplex real-time PCR assay developed in this study could detect up to 0.54±0.09 and 0.65±0.07 log10 cfu/ml for *S.*Typhimurium and *S.*Enteritidis for beef, 1.45±0.21 and 1.65±0.07 log10 cfu/ml for *S.*Typhimurium and *S.* Enteritidis for pork, respectively, with all conditions optimized (Lee et al. 2009). Miller et al. (2011) applied real-time reverse-transcriptase-PCR (rt-T-PCR) to detect *Salmonella* from spiked lettuce and tomatoes within one day. Twenty-five grams of lettuce and ~100 g of tomatoes were inoculated with 1-8 log cfu of an overnight culture of *S.*Typhimurium.

4.4 LAMP

The loop-mediated isothermal amplification (LAMP) assay is a rapid, specific, and sensitive method that has potential application for routine diagnostics of food pathogens. This isothermal method does not require expensive equipment such as a PCR thermocycler but only a simple waterbath for amplification within 90 min. Detection is even simpler by visual eye or turbidimeters that are less expensive than fluorescent spectrophotometers or real-time PCR machines. All these advantages make it a practical approach for routine use

by processing industries to rapidly detect *Salmonella* in their environment and to implement appropriate control strategies.

The 220 strains of 39 serotypes of *Salmonella subsp. enterica* and 7 strains of *S.*enterica subsp. *arizonae* were amplified. The sensitivity of the LAMP assay was found to be >2.2 cfu/test tube using nine serotypes (Hara-Kudo et al. 2005). Techathuvanan et al. (2010) presented a LAMP method for the rapid and sensitive detection of *S.*Typhimurium from pork. This LAMP assay using 6 specific *invA* gene primers and *Bst* DNA polymerase reaction mix was carried out at 62℃ for 90 min in a waterbath. Turbid products were detected visually and by agarose gel electrophoresis. Improved *Salmonella* detection at 10^2 cfu/25g for both pork chop and sausage was obtained after 10-h enrichment and 10^6 cfu/25g without enrichment for both products. Chen et al. (2011) examined a novel strategy for the rapid detection and quantification of viable *Salmonellae* in produce by coupling a simple propidium monoazide sample treatment with loop-mediated isothermal amplification (PMA-LAMP). Under the optimized condition, PMA-LAMP consistently gave negative results for heat-killed Salmonella cells with concentrations up to 10^8 cfu/ml. The detection limits of PMA-LAMP were 3.4 to 34 viable *Salmonella* cells in pure culture and 6.1×10^3 to 6.1×10^4 cfu/g in spiked produce samples. The correlation between LAMP time threshold values and viable *Salmonella* cell numbers was high (R^2= 0.949 to 0.993), with a quantification range (10^2 to 10^5 cfu/reaction in pure culture and 10^4 to 10^7 cfu/g in produce) comparable to that of PMA in combination with quantitative real-time PCR.

5. BIOSENSORS

An electrochemical immunosensor based on screen-printed gold working electrode with onboard carbon counter and silver-silver chloride pseudo-reference electrode for *Salmonella* Typhimurium detection was described by Salam and Tothill (2009). Villamizar et al. (2008) reported a fast, sensitive and label-free biosensor for the selective determination of *Salmonella* Infantis. It is based on a field effect transistor (FET) in which a network of single-walled carbon nantotubes (SWCNTs) acts as the conductor channel.

Ravindranath et al. (2011) demonstrated a cross-platform approach to simultaneously detect three different pathogens using Raman and UV-vis absorption spectroscopy. Gold (Au), silver (Ag), and Ag-Au core-shell nanoparticles were functionalized with anti-Salmonella typhimurium aptamers, anti-Staphylococcus aureus and anti-*Escherichia coli* O157:H7 antibodies respectively and labeled with unique Raman reporter molecules. A microfiltration step was used to consolidate a highly selective and specific detection platform, with total detection time under 45 min for both species (*E. coli* O157:H7 vs. *S.typhimurium*) and strain (*E.coli* O157:H7 vs. *E.coli* K12) level sensing at a limit of a detection, ranging between 10^2 and 10^3 cfu/ml. This simple yet robust multiplex detection platform has the potential to be developed into a rapid and portable pathogen sensor for ultrasensitive detection in liquid samples. A colorimetric method for assay of *E.coli* O157:H7 was developed as a model for gram-negative bacteria by a mercaptoethylamine-modified gold nanoparticles (MEA-AuNPs) sensor (Su et al. 2012). Mercaptoethylamine (MEA) molecule binded with *E. coli* O157:H7 via electrostatic adhesion between the positive electricity of MEA and the negative electricity of *E. coli* O157:H7. Wang et al. (2011) developed a multiplex immunoassay by integrating magnetic nanobeads (MNBs) for immunoseparation with quantum dots (QDs) as fluorescent labels for rapid, sensitive, and simultaneous detection of three major pathogenic bacteria, *S.*Typhimurium, *E.coli* O157:H7, and *L.monocytogenes*, in food products.

6. CONCLUSIONS

Many high-risk pathogens that cause disease in humans are transmitted through various food items. Due to increased morbidity and mortality leading to time lost in the work place and reduced productivity, foodborne disease across the world costs billions of dollars annually and constitutes a major public health problem (Naravaneni and Kaiser, 2005). One of the inherent difficulties in the detection of food pathogens is that they are generally present in very low numbers in the midst of up to a million or more other bacteria. These microbes may be lost among a background of indigenous microflora, and substances in the foods themselves may hinder recovery. There is also the difficulty of demonstrating that the strains recovered from a food sample are, indeed, pathogenic to human beings (Sockett, 1991).

The conventional microbiological methods for detection of these bacteria, however, usually include multiple subcultures and biotype- or serotype-identification steps and, thus are laborious and time-consuming. Rapid and easy detection of pathogenic organisms will facilitate precautionary measures to maintain healthy food. With continued development of novel technologies for rapid, high-throughput detection of foodborne pathogenic bacteria, a number of unique approaches and variations on existing techniques will be investigated and implemented with many advatanges such as more ease of use, more reliability and lower cost.

7. ACKNOWLEDGEMENT

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