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Review

Polymerase chain reaction-based serotyping of pathogenic bacteria in food

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ABSTRACT

Serotyping analysis of bacterial pathogens in food products is important for foodborne disease surveillance and outbreak investigations. Traditional immunological techniques are labor-intensive and time-consuming, whereas polymerase chain r eaction (PCR)-based techniques are more robust, consistent and rapid. PCR-based methods also provide easier standardization and better reproducibility. Here, we summarize some recent developments and applications of PCR-based serotyping for common foodborne pathogens, and provide a list of available bioinformatics tools for developing PCR-based serotyping assays.

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1. Introduction

Approximately 10 million cases of foodborne illnesses that are caused by 31 known pathogens occur annually in the United States (CDC, 2011; Scallan et al., 2011a,b). Bacterial pathogens account for approximately 42% of these cases, with the remainder linked to norovirus, parasites, and chemicals. The top-ranked bacterial pathogens linked to the majority of foodborne disease outbreaks include: *Salmonella* spp., *Staphylococcus* spp., *Shigella*, *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter* spp. and *Clostridium* spp. In 2013, FoodNet identified approximately 19,000 cases of infections, 4200 cases of hospitalizations, and 80 deaths due to foodborne bacterial pathogens (Crim et al., 2014). However, since most illnesses in healthy individuals are self-limiting and not reported, the actual number of cases of illnesses is undoubtedly much higher (Crim et al., 2014).

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Foodborne illnesses associated with bacterial pathogens are of significant public health concern. Serotyping of these pathogens provides important information for tracking the origins of contamination. For example, different *Salmonella* serovars are generally associated with different hosts or geographical locations. The serotype information also provides insight into the type of disease likely to occur, the severity, and the potential antimicrobial resistance of the pathogen (Ranieri et al., 2013). Serotyping analysis is typically performed in an outbreak investigation not only for identifying the cause of infection but also for identifying epidemiological sources. Traditional serotyping techniques, or phenotype-based assays, are routinely used for this purpose. Recently, however, there has been an increase in the application of PCR-based, or DNA-based, serotyping methods in foodborne disease outbreak investigations. In this review, we compare traditional serotyping techniques and PCR-based techniques, and provide a summary of the advantages and applications of, and available bioinformatics tools for PCR-based assays for select foodborne bacterial pathogens.

2. Basics of traditional serotyping technique

Traditional serotyping techniques have been used for years and worldwide, which contribute to the identification and surveillance of bacterial pathogens in food products and associated human foodborne outbreaks (Cheng et al., 2014; Wattiau et al., 2011). In traditional serotyping, antigens, such as components of the outer wall of bacterial pathogens, are detected by agglutination of bacterial cells with specific antisera (Mohan and Kumar, 1989; Owen et al., 1994; Prager et al., 2003; Schrader et al., 2008; Selander et al., 1996). Targeted antigens include bacterial somatic (O), flagellar (H), and capsular (Vi) proteins. O antigen is generally used to serotype Gram-negative bacterial pathogens due to the high diversity of the protein (Reeves and Wang, 2002; Sun et al., 2011). The Kauffmann–White scheme defines *Salmonella* serotypes based on their specific antigenic formulations and has been an important method for identifying strains for both epidemiological purposes and disease surveillance (Popoff et al., 2001; Poppoff and Le Minor, 2001; Sun et al., 2011).

Traditional serotyping, despite its wide use, has a number of disadvantages: it is time consuming, laborious, and can be imprecise (Blackburn, 1993; Borucki and Call, 2003; Doumith et al., 2004; Ranieri et al., 2013; Swaminathan and Feng, 1994). Serotyping often results in 5 to 8% of isolates being partially or completely untypable (Kim et al., 2006). The typing also requires the maintenance, storage, and quality control of hundreds of specific sera and antigens (Guibourdenche et al., 2010; Ranieri et al., 2013). Therefore, standardization is often difficult among laboratories for the same bacterial pathogens. These disadvantages can delay the submission of information to public health data information systems (McQuiston et al., 2011). Therefore, there is a strong need for the development and use of more rapid and standard methods for the determination of serotypes of foodborne bacterial pathogens.

3. PCR-based serotyping technique

Polymerase chain reaction (PCR) has become the most frequently used method for amplifying nucleic acids since it was developed by Mullis and coauthors (Mullis et al., 1986; Mullis, 1990). The reaction system includes a DNA polymerase, template DNA from the pathogen(s) under investigation, and two complementary oligonucleotide primers designed to amplify the sequence of the template DNA. This approach has been frequently used for a growing number of serotypic studies to detect and characterize bacterial foodborne pathogens. The development of PCR-based serotyping assays typically includes steps of mining and identifying serotype-specific genomic markers, designing PCR primers, validating the specificity of the assay using reference bacterial strains, and testing and optimizing the PCR assays for detection in broth cultures and food samples. For a comparison of traditional and PCR-based serotyping methods, see Table 1.

PCR-based serotyping techniques, unlike traditional methods, are advantageous in that they provide concise results and have better standardization and reproducibility among laboratories (Barco et al., 2011; Herrera-Leon et al., 2007). Detection targets are often specific DNA sequences in the genome or a plasmid of a bacterial pathogen, which encode antigens or serotype-specific proteins. Traditional serotyping techniques require identification of antigens, such as O, H, and Vi antigens; the specific combinations of these antigens can distinguish between bacterial species and their serotypes; whereas PCR-based strategies can often be accomplished using DNA sequences which are associated with these antigens (Kerouanton et al., 2010; Mortimer et al., 2004; Ranieri et al., 2013; Yang et al., 2007). A number of PCR-based serotyping assays have been developed in recent years, including some real-time PCR and multiplex PCR assays for specific bacterial foodborne pathogens (Table 2). These methods were designed to both identify bacteria and their serotypes rapidly and efficiently. For example, a multiplex PCR assay was developed to determine *Salmonella* serotypes via antigenic determination of five O antigens, eight H1 antigens, and seven H2 antigens (Herrera-Leon et al., 2007). PCR-based methods can be used with heterogeneous samples, such as contaminated food and food products.

With the increasing and routine application of whole genome sequencing technologies, numerous foodborne bacterial pathogens of various serotypes have been sequenced. Using comparative genomic analysis, serotype-specific regions, or markers, within a genome can be identified (Arrach et al., 2008). For example, a multiplex PCR assay was developed using specific sequence markers to distinguish between seven serotypes of *Salmonella* (Akiba et al., 2011); such assay was able to differentiate these serotypes with 100% accuracy. The PCR results are easy to interpret based on the presence or absence of an amplicon band. The development of newer high-throughput genomic sequencing technologies has led to an exponential increase of the whole genome sequences of various foodborne pathogens. These sequences can be utilized for the identification of novel serotype-specific DNA markers to aid in designing PCR-based serotyping schemes.

3.1. Salmonella enterica

S. enterica is the causative agent of human salmonellosis and one of the most prominent foodborne pathogens worldwide. This pathogen is commonly transmitted via consumption of contaminated eggs, dairy, vegetables, and processed foods or by contact with infected animals, such as reptiles and birds (Hoelzer et al., 2011). Methods which can differentiate *S. enterica* beyond the species level (e.g., to over 2500 known serotypes, of which 1478 belong to *S. enterica* (Porwollik and McClelland, 2003)) are essential in mitigating and controlling this bacterial pathogen (Guibourdenche et al., 2010; Olaimat and Holley, 2012; Shi et al., 2013).

Several PCR-based assays to detect *S. enterica* serotypes have been developed, which target either antigen-related genes or serotype-

Table 1
Comparison between traditional and PCR-based serotyping techniques.

	Traditional serotyping	PCR-based serotyping
Target	Cell surface antigen proteins	Antigen-coding genes or serotype-specific genes not directly related to surface structures, or serotype-specific, noncoding genomic markers
Required materials	Antisera	Primers
Prerequisite assay	Isolation of target organism Antibody–antigen agglutination	PCR reagents Genomic DNA extraction Regular PCR, multiplex PCR, or real-time PCR
Confirmation	Visual	Gel electrophoresis or DNA sequencing or real-time PCR Ct values