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Review

Nucleic Acid-based Biotechnologies for Food-borne Pathogen Detection Using Routine Time-intensive Culture-based Methods and Fast Molecular Diagnostics

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Abstract Diseases caused by food-borne pathogens constitute a major burden to consumers, food business operators, and national governments. Bacterial and viral pathogens are the major biotic factors influencing food safety. A vast array of culture dependent analytical methods and protocols have been developed. Recently, nucleic acid-based methods have begun to replace or complement culture-based methods for routine use in food control laboratories. Basic advantages provided by nucleic acid-based technologies are faster speed and more information, such as sub-species identification, antibiotic resistance, and food microbiology. In particular, PCR and alternative methods have been developed to a stage that provides good speed, sensitivity, specificity, and reproducibility with minimized risk of carryover contamination. This review briefly summarizes currently available and developing molecular technologies that may be candidates for involvement in microbiological molecular diagnostic methods in the next decade.

Keywords: food, diagnostic, pathogen, detection, biotechnology

Introduction

Food safety issues occur repeatedly in many countries and more and more consumers and organizations worldwide are paying attention to food safety. Numerous outbreaks of bacterial and viral pathogens can be linked to consumption of different foods. In order to ensure the safety of food and to reduce consumer concerns, many countries have established early warning systems as a means of prevention and control of food-borne diseases. Such systems enable checks at every segment of the chain from food production, processing, marketing, and surveillance. The key step in setting up such systems is use of high efficiency food safety testing methods, which have to be accurate and sensitive enough for detection of low levels of contamination in commodities for prevention of pathogen transmission through the food chain to human or animal consumers (1).

Microbiological analysis is an integral part of technological food safety quality control and monitoring systems, and of management of microbial safety in the food chain. Analysis is also an important tool for investigation of critical control points and for evaluation of good hygienic practices and standard operating procedures (2,3). Traditional microbiological analytical methods for pathogenic bacteria and viruses in foods, which constitute the basis of current standard methods, involve isolation based on culturing. The entire procedure requires several days, but is generally reliable and analytical parameters are well defined. However, due to time requirements, these methods are often ineffective due to incompatibility with the speed at which food products are manufactured and with product shelf life. In order to meet requirements for faster analysis, several rapid methods that are faster than classical counterparts have been developed (4,5).

Nucleic acid-based technology is a valuable molecular tool for basic research and application oriented fields, such as clinical medicine, infectious disease identification, gene cloning, and industrial quality control. These molecular methods were previously considered unsuitable for routine testing of food products for pathogenic contamination because these techniques were only useful in research laboratories with skilled technicians (6). However, in the last decade, nucleic acid-based methods have gradually started to replace or complement culture-based methods and immunochemical assays in routine laboratory analysis for food control.

Recent nucleic acid-based technologies that can be used for detection of viral and bacterial food-borne pathogens in food control laboratories are reviewed herein. Principles, applications, challenges, prospects, and limitations of these methods and considered. PCR

was the first nucleic acid-based technology to be used. Several additional methods have been developed, such as strand displacement amplification, loop mediated isothermal amplification, and nucleic acid sequence based amplification. These novel techniques, which can be applied when PCR technology application is limited, offer high sensitivity and specificity for food-borne pathogen detection. Nevertheless, these methods need to be validated before use.

Nucleic acid-based tools for identification of food-borne viruses

Viruses are a leading cause of food-borne illness and food-borne outbreaks worldwide (7). Although a large variety of food-borne viruses exist, the norovirus (NoV) and the hepatitis A virus (HAV) are the most important owing to frequent occurrence and severity of related illness, respectively (8). Other food-borne viruses include, but are not restricted to, rotavirus, sapovirus, adenovirus, astrovirus, hepatitis E virus (HEV), and aichivirus (9). The majority of food-borne viruses cause gastro-enteritis, but HAV and HEV can cause hepatitis. Studies have been performed for screening of viral contamination of food products. Most food-borne viruses are difficult or currently impossible to cultivate (10), which has resulted in use of sensitive molecular methods for detection in foods. In general, molecular detection of viral contamination requires a detection strategy involving 3 successive steps. In the first step, the virus is extracted from a food matrix and concentrated in a small volume. Viral nucleic acids are subsequently purified from virus extracts in the second step using a variety of protocols. Finally, in the third step, specific viral sequences are detected in purified nucleic acids using molecular methods (11,12). Some of these nucleic acid-based technologies for screening of viral food contamination are presented herein.

Polymerase Chain Reaction (PCR) PCR is a widely used molecular tool for screening of food-borne viruses. This technique consists, briefly, in amplification of DNA or RNA fragments following a thermal profile consisting of the 3 steps of (i) heat denaturation of double-stranded DNA, (ii) annealing of 2 primers with the DNA matrix using an appropriate hybridization temperature, and (iii) extension of primers for synthysis of target DNA (13) (Fig. 1).

The PCR product, called the amplicon, can be detected using a variety of methods and can be identified by size, sequence, chemical modification, or a combination of these parameters. Characterization by size can be achieved using gel electrophoresis with agarose or polyacrylamide gels or capillary electrophoresis, or using column chromatography. Characterization by sequence composition can be achieved based on specific hybridization of probes having a sequence complementary to the target sequence, or by cleavage of amplified material reflecting target-specific restriction-enzyme sites. Characterization by chemical modification can be achieved based on incorporation of a fluorophore into amplicons and subsequent detection of fluorescence following excitation. Detection of amplicons can also be achieved using probes labeled to allow subsequent radioisotopic or immuno-enzyme-coupled detection (14).

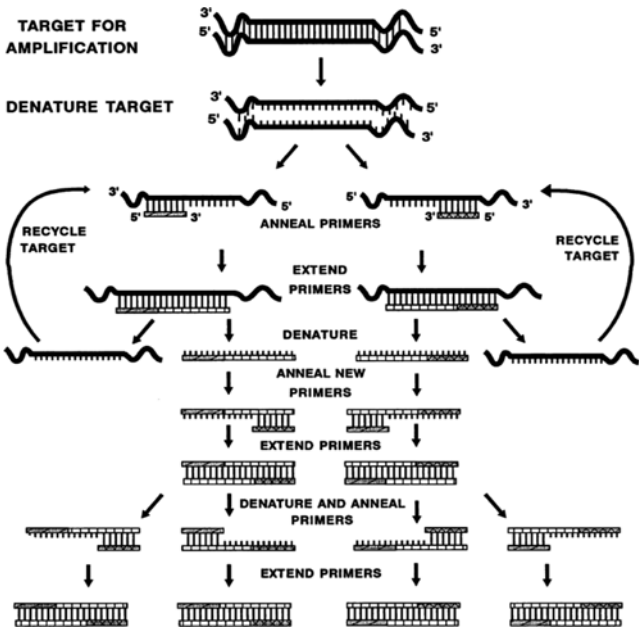

Fig. 1. The PCR amplification procedure (adapted from Wolcott (76) with permission of the publisher).

Multiplex-PCR An important cause of food-borne diseases is human enteric viruses, such as noroviruses and the hepatitis A virus (15). Vegetables, fruits, meats, shellfish, and sandwiches are the most implicated foods (16-18). Limitations in detection methods for these viruses in food products have raised demand for development of efficient standard isolation and identification techniques. Consequently, molecular nucleic-acid based technologies provide solutions to allow genetic characterization of these viruses. Reverse transcription (RT)-PCR, was first applied using a monoplex method, followed by development of a multiplex RT-PCR system (Fig. 2) that was used by Rosenfield and Jaykus (19) for simultaneous detection of human enteroviruses, the hepatitis A virus, and the Norwalk virus. In the same context, Beuret (20) reported a multiplex real-time method for detection of genogroups I and II noroviruses, human enteroviruses, and astroviruses. Few studies have used RT-PCR methods for detection of enteric viruses in environmental and food samples (21). While shellfish has been the most common food commodity explored, a few studies have reported RT-PCR detection of viruses in produce and ready-to-eat foods (18,22,23).

Nucleic Acid Sequence-Based Amplification (NASBA) Nucleic acid sequence-based amplification (NASBA) is an isothermal nucleic acid amplification method that amplifies an RNA template (Fig. 3) (24-26). This technique is applied for detection of RNA viruses, such as the hepatitis A virus (27) and noroviruses (28). Additionally, Greene *et al.* (28) and Lunel *et al.* (29) reported that the NASBA amplification efficiency is comparable to or better than the efficiency of RT-PCR.

Comparative analysis of RT-PCR and NASBA showed that NASBA has advantages in specificity and sensitivity. In fact, NASBA is more

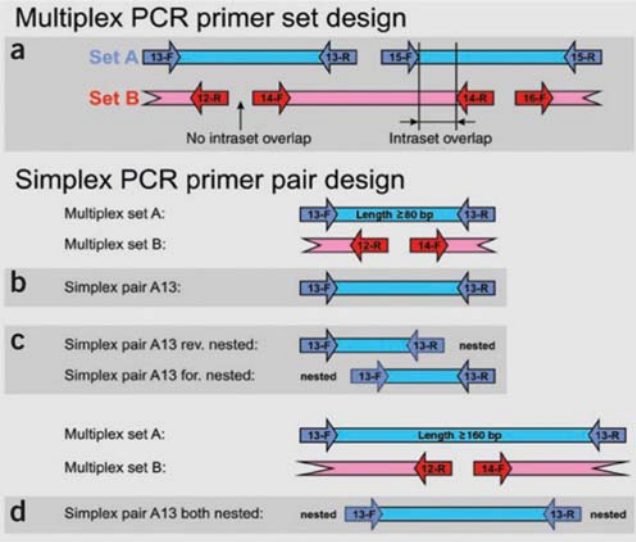

Fig. 2. Multiplex DNA amplification strategy (adapted from Römpler *et al.* (77) with permission of the publisher).

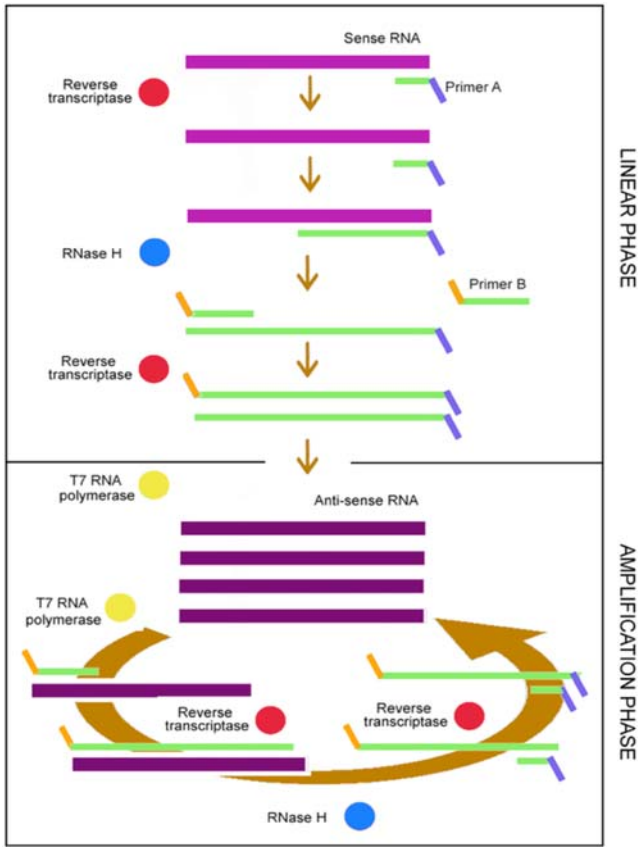

Fig. 3. Schematic representation ofnucleic acid sequence-based amplification (NASBA) (adapted from Fusco and Quero (25) with permission of the publisher).

suited for detection of RNA viruses than RT-PCR since it is an RNA amplification method. Although the same numbers of copies are generated using both methods, this number is achieved in a shorter time using NASBA (30). Additionally, NASBA does not require any denaturation step.

Jean *et al*. (18) also developed a multiplex nucleic acid sequence-based amplification method for specific, simultaneous, and rapid detection of epidemiologically relevant human enteric viruses. Once developed and optimized, the assay was applied for detection of virusesin model ready-to-eat foods artificially seeded with viral contamination. In experiments performed using viral stock cultures alone, monoplex NASBA detection limits were similar to limits reported for more established amplification methods, such as RT-PCR, in agreement with Greene *et al*. (28) regarding the detection limit for the genogroup I norovirus. In the multiplex format, an approximately 10x decrease in detection sensitivity was reported (18).

For pathogen detection, NASBA has a theoretically higher analytical sensitivity than RT-PCR, making it an established diagnostic tool (31). NASBA has potential for detection and differentiation of viable cells via specific and sensitive amplification of messenger RNA, even against a background of genomic DNA (32,33). However, NASBA is not an ideal method and has limitations. A low thermal profile of 41°C raises the risk of non-specific amplification. Also, NASBA does not allow further genetic analyses, such as sequencing, because the final product of the reaction is RNA.

Quantitative PCR (qPCR) Quantitative PCR (qPCR) is rapid, sensitive, and reproducible, compared with the above mentioned amplification methods (Fig. 4). Furthermore, it is less affected by carryover contamination as specific amplicons are detected in a closed tube. Another advantage of this technology is elimination of the need for different rooms for post-PCR processing, and no need for use of mutagenic ethidium bromide (34,35). Methods based on simplified enrichment coupled with real-time PCR represent an important developmental step because they are clearly faster by up to several days than classical counterparts. Method analytical parameters are well defined and technically developed to a level that allows routine use. Furthermore, these techniques are time-saving and cost-effective (36). However, novel amplification technologies have not been implemented in diagnostic food laboratories due to lack of validation and standardization.

qPCR results require correct technical interpretation. Use of appropriate controls should be included and respected when testing foods for viral contamination (37). Use of negative controls is particularly important owing to the high sensitivity of qPCR and a potential risk for crossover or carryover contamination during application of PCR methods in a laboratory (38). Furthermore, experienced scientific personnel are required to correctly analyze qPCR results. Limitations of qPCR should be considered when interpreting positive results during testing of foods for food-borne viral presence. Most importantly, qPCR is an amplification based molecular method for detection of viral nucleic acids and is,

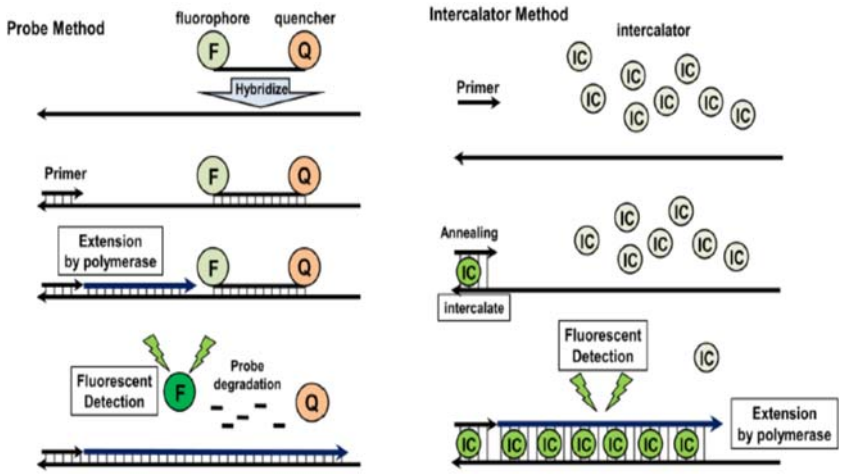

Fig. 4. Schematic diagram of quantitative real-time PCR (qPCR). This method uses molecular oligonucleotide probes conjugated with a fluorescent molecular or chemical dye for staining of PCR products (adapted from Sakurai and Shibasaki (78) with permission of the publisher).

therefore, not suitable for differentiation between infectious and non-infectious viral particles (39). Reasons for this limitation are (i) amplification of viral genomic material does not provide information regarding the integrity of the virus capsid, (ii) the persistence of detectable viral RNA in the environment, and (iii) detection of a small genomic fragment does not give information regarding the integrity of the viral genome (12,40). However, owing to a high sensitivity, qPCR remains a standard for detection of viral contamination in food products.

Nucleic acid-based tools for identification of pathogenic food-borne bacteria Numerous outbreaks of the bacterial pathogens *Escherichia coli, Salmonella* spp*., Shigella* spp*., Yersinia enterocolitica, Brucella* spp*., Campylobacter jejuni, Cronobacter sakazaki, Clostridium* spp*., and Staphylococcus aureus,* and others can be linked to consumption of different foods worldwide. In order to improve food safety during all stages of food production, a hazard analysis critical control points (HACCP) system should be implemented for the production chain. Classical culture based methods, which are time and cost-intensive, remain necessary, especially for viable and cultivable pathogens (1).

The classical conventional method for food-borne pathogen detection requires plating food samples on selective and non-selective media, followed by enumeration of pathogenic bacteria. Nevertheless, in some cases where metabolic products (enzymes, toxins) or cellular components (endotoxins) are responsible for clinical symptoms, culture-based methods fail to detect a viable pathogen. In addition, culture-based methods often require an enrichment step (1), which constitutes another disadvantage. Consequently, classical techniques are more and more being replaced by faster molecular nucleic acid-based technologies.

Multiplex PCR *In vitro* amplification of nucleic acids using PCR has become a powerful diagnostic tool for analysis of bacteria in foods (25). PCR is a technique that has allowed identification and molecular typing of several pathogenic bacteria. Fully completed PCR protocols have been reported and validated for identification of bacteria in foods (34,35). Nevertheless, this method has limitations of (i) requirement of an enrichment step, (ii) use of gel electrophoresis for detection of a PCR product, and (iii) a detection limit of approximately 10^2-10^3 CFU (colony forming units)/mL (6).

Multiplex PCR protocols combining both enrichment and amplification reaction steps are time-saving and cost-effective alternatives for food-borne pathogen identification in only a single assay. In fact, multiplex PCR allows detection of many specific DNA markers in the same reaction and under specific experimental conditions (13,41-43).

DNA probes can be labeled using different fluorescent dyes allowing discrimination of individual targets without markers and cross-talk, which is not the case for the simple PCR reaction. In this context, a quantitative PCR strategy allows independent detection of several dyes in a reaction mixture. However, detection must be performed using a spectrograph or individual channels containing narrow-band excitation and filters for emission (36,44).

Amplification of ribosomal RNA (rRNA) genes The major product of all cellular transcription in both prokaryotes and eukaryotes is ribosomal RNA (rRNA). In most prokaryotes, rDNA forms an operon with an order of 16S-23S-5S and is co-transcribed in a single polycistronic RNA that requires processing for generation of RNA species present in the mature ribosome (45). Comparative sequence analysis of rDNA has been widely used to infer phylogenetic relationships. In addition to phylogenetic aspects, structural studies of rRNAs are of fundamental importance for understanding the topology and function of ribosomes (46,47). Furthermore, rDNA molecules contain signature structures, which are unique for groups of organisms, that render rDNA as ideal targets for specific nucleic acid probes (48,49).

During the last decade, biologists have used a variety of molecular

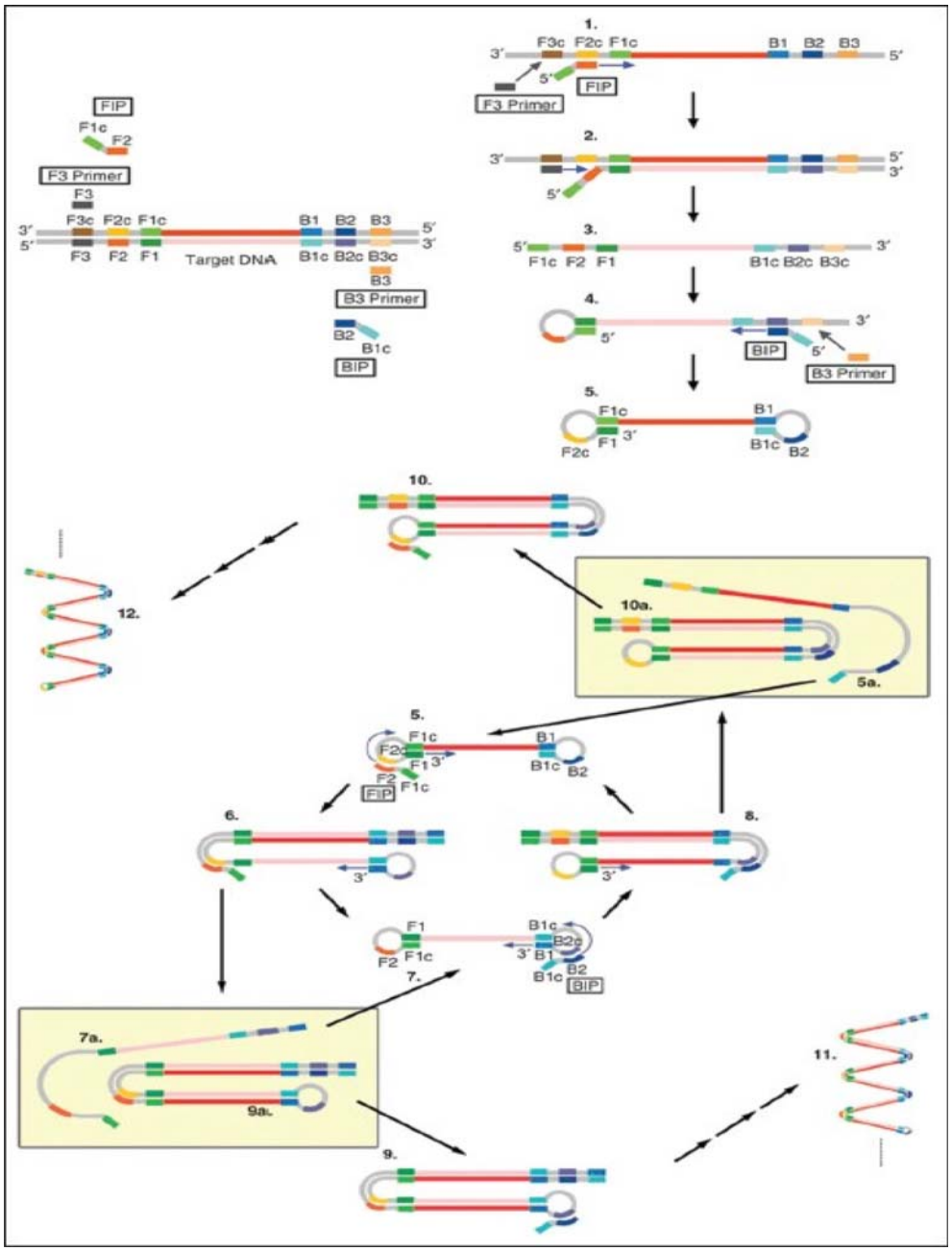

Fig. 5. Schematic description of the loop mediated isothermal amplification assay (LAMP). The mechanism of the LAMP amplification reaction includes 3 steps of production of starting material, cycling amplification, and elongation and recycling (adapted from Fakruddin *et al.* (33) with permission of the publisher).

techniques based mainly on ribosomal DNA (rDNA) genes to identify microorganisms. In fact, many studies based on sequences of 16S and 23S rDNA have been carried out (25,50-52). However, due to reduced selection pressure, the 16S-23S rDNA intergenic spacer region sequence seems to be more genetically variable and species specific than the 16S and 23S rDNAs regions (50,53). In addition, variation in lengths and sequences of these spacer regions suggests use for design of genus or species specific DNA probes (53). It has been reported that helices 45 and 54 (*Escherichia coli* numbering) are the most variable regions in 23S rDNA (49).

Salmonellosis is one of the most widespread food-borne bacterial illnesses in humans. The most frequently isolated serovars from food-borne outbreaks are *Salmonella enterica* serovars Typhimurium and Enteritidis (49,54,55). Conventional methods for detection of *Salmonella* require multiple subculture steps, followed by biochemical and serological confirmation, which may require 4-6 days. More than 2,500 *Salmonella* serovars have been identified (56). Thus, rapid and efficient methods for detection of *Salmonella* serovars are required. It has been recently reported that 23S rRNA genes are useful for definition of serovar-specific probes for detection of *Salmonella* in foods (49).

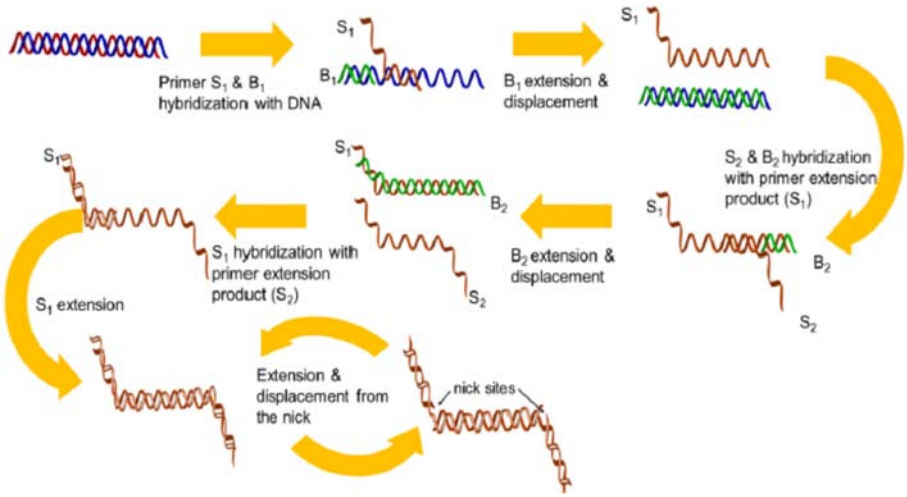

Fig. 6. Schematic representation of the strand displacement amplification (SDA) reaction (adapted from Chang *et al.* (60) with permission of the publisher)

Quantitative PCR (qPCR) Although providing rapid and specific results, PCR-based methods require dedicated laboratory equipment, which makes these methods expensive and time-consuming. Quantitative real-time PCR (qPCR) assays are faster, as compared to conventional PCR, and eliminate gel electrophoresis based on detection of fluorescence during DNA amplification (1,43,57).

qPCR is a challenging technique where gene amplification occurs during a reaction. This technique has advantages of (i) providing quantitative gene expression DNA copy numbers, (ii) minimization of contamination risks since the reaction takes place in a closed tube, and (iii) no use of a detection protocol, such as gel electrophoresis. Many commercial qPCR kits are available, such as TaqMan, SybrGreen, and FRET probes (36).

qPCR has been useful for detection of several pathogenic bacteria in foods. Nevertheless, since the detection limit is approximately 10^2-10^3 CFU/mL, qPCR requires enrichment steps (5,58) and requires sophisticated and expensive equipment that is not readily available for routine detection in processing facilities and small industrial laboratories. Moreover, PCR and qPCR both require use of a pure template, thus requiring labor and cost intensive extraction and template DNA cleanup procedures (1).

New alternative amplification technologies With development of primer initiated enzymatic *in vitro* nucleic acid amplification technologies, protocols for specific detection and identification of microorganisms directly from foods were developed (1,25,26,36, 43,59-62). Alternative nucleic acid based technologies, called isothermal amplification techniques since they require a constant temperature, are attracting more and more interest for bacterial food-borne analysis. These recent methods are technically simple, time-saving, and cost-effective.

Several isothermal amplification techniques, such as loop mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), isothermal amplification with continuous luminometry, rolling circle amplification (RCA), isothermal multiple displacement amplification (IMDA), and others have been proposed for molecular identification and typing of food-borne pathogens. None of these non-PCR-based methods require use of a thermocycler, which represents a positive feature. Some of these recent techniques are discussed below.

Loop-Mediated Isothermal Amplification (LAMP) Loop-mediated isothermal amplification (LAMP) is a relatively novel method that amplifies nucleic acids under isothermal conditions (1,26,43,62,63). It has been successfully applied for detection of *Escherichia coli* (64), *Salmonella* (65), and *Vibrio parahaemolyticus* (66).

LAMP has advantages of (i) technical simplicity at a constant temperature of 60-65°C, (ii) time-saving with processing in less than 1 hour), (iii) cost-effectiveness using a simple heating block, and (iv) insensitivity to inhibitors or contaminants and, therefore, does not require extensive DNA purification prior to amplification (67) Four primers recognizing 6 separate regions within a target DNA are used in this method and the amplification reaction occurs only when primers correctly recognize all 6 regions (Fig. 5). Consequently, this method requires correct design of oligonucleotides and leads to higher specificity than classical PCR (36,68). LAMP is more resistant than PCR to inhibitory compounds present in analytical samples, eliminating need for extensive DNA purification. Based on a combination with reverse transcription, LAMP can amplify ribonucleic acid sequences with high efficiency. LAMP is a specific and sensitive technique (63) that allows genetic processing of amplicons based on sequencing or pyrosequencing (33,69,70).

Strand Displacement Amplification (SDA) Strand Displacement Amplification (SDA) is another isothermal amplification technique (26,33) that requires 4 primers to hybridize with a target DNA sequence that contains a specific restriction site for exonuclease HincII (Fig. 6). The reaction consists briefly in (i) annealing of the

Table 1. Summary of nucleic acid-based technologies used for food-borne pathogen detection

Nucleic-acid based methods	Advantages	Disadvantages	Applications	Key References
RT-PCR Multiplex PCR	Simple method Simplicity of primer design Production of a large number of DNA copies	Extraction and denaturation steps required Risk of non specific amplification or contamination Costs and time requirement	Genomic RNA or DNA of bacterial and viral pathogens	Mullis (74) Römpler *et al.*(77) Rosenfield and Jaykus (19)
qPCR	Simplicity of primer design Rapidity, sensitivity and reproducibility No additional processing of the PCR product Provides quantitative data	Labor and cost-intensive extraction Denaturation step required Necessity of template purity Risk of crossover and carryover contamination Sophistical and expensive equipment required Costly method	Detection of viral and bacterial contaminants in food products Quantitative analyses	Fratamico *et al.* (41) Rodríguez-Lázaro *et al.* (35)
NASBA	Simplicity of primer design No need for a denaturation step Rapidity of amplification Higher analytical sensitivity than PCR	Non-ideal for DNA detection and analysis Requirement of an RNA template Possibility of non specific contamination due to a low incubation temperature (41°C)	RNA of many bacterial species (*Mycobacterium tuberculosis*…), viral RNA (Enteric viruses…)	Compton (24) Fakruddin *et al.* (32) Jean *et al.*(18)
LAMP	High specificity and efficiency of amplification Rapidity under isothermal conditions No need for DNA purification Cheapest compared to PCR methods	Complexity of primer design Less efficient for short targets	Viral and bacterial food-borne pathogen detection	Niessen *et al.* (1) Notomi *et al.*(63) Saharan *et al.* (62)
SDA	Rapidity of the reaction Sensitivity and efficiency Possible semi-quantitation	Complexity of primer design Necessity of sample preparation Less efficient for amplification of long target sequences	Genomic DNA of *Mycobacterium* species and others	McHugh *et al.* (72) Walker *et al.* (71)

PCR, Polymerase Chain Reaction; RT-PCR, Reverse Transcription PCR; qPCR, Quantitative PCR; NASBA, Nucleic Acid Sequence Based Amplification; LAMP, Loop-MediatedIsothermal Amplification; SDA, Strand Displacement Amplification

primer to a displaced target DNA, (ii) extension using exo-Klenow, (iii) nicking of the HincII site, and (iv) final extension of the nicked fragment (71). SDA produces in a single reaction approximately 10^9 copies of target DNA in less than an hour (72). Several SDA commercial kits are available, including BDProbeTec (Becton Dickinson, Franklin Lakes, NJ, USA), that had been used for identification of *Mycobacterium tuberculosis* (72). However, a major limitation of SDA is lack of efficient amplification of long target sequences (33).

Isothermal amplification with continuous luminometry Isothermal amplification with continuous luminometry is another isothermal amplification strategy that occurs in a closed tube placed in a thermostated luminometer. The technique consists, briefly, in reacting labeled primers with inorganic PPi and *Bst* DNA polymerase in a closed tube system incubated at 60°C for 75 min (36). When the reaction occurs, PPi is released during generation of ATP. Kits for the detection of *Escherichia coli* O157*, Salmonella* and *Listeria* are currently available on commercial basis.

Kuchta *et al*. (36) reported that isothermal amplification with continuous luminometry has the same sensitivity and detection limit as a quantitative PCR assay coupled with enrichment. However, some commercial protocols are not genus specific (73) and need to be proven and validated. Specific genetic markers can be selected and optimized within these protocols in order to gain a better specificity of performance, which is, compared to quantitative PCR, less efficient for analytical parameters and specificity of detection.

Based on these data and taking into account that the microbiological identification is often required for confirmation of suspicions concerning infections, microbiological diagnostic tools must be efficient and powerful, particularly for food-borne infections. Traditional methods for microorganism identification require days and cannot satisfy the need for rapid testing. Gram staining and cell cultures have been traditionally used for detection of bacterial and viral pathogens, respectively, in general requiring 48 to 96 h for identification. Thus, new technologies are needed to shorten the time for detection and identification.

Nucleic acid-based technologies are novel alternatives specifically based on molecular biology and biotechnology tools and are useful in many areas, such as agriculture, medicine, research, and laboratory analysis. PCR, the first developed method (74), remains an

Table 2. Examples of application and improvement of nucleic acid-based techniques for food-borne pathogen detection

Nucleic-acid based methods	Food matrix	Detected food-borne pathogens	Detection limit and improvement	Assay time	References
RT-PCR Multiplex PCR	Milk, cheese, butter, chicken, eggs, carcasses, sandwiches, ready to-eat- foods contaminated pork	*L. monocytogenes,* *S.* spp.*,* *S. aureus,* *E. coli*	10^2-10^3 CFU/mL	24 h	Amaglian*iet al.* (59) Souii *et al.* (49)
qPCR	Fruits, vegetables, contaminated chicken, eggs, meat, milk, fresh pork, dairy products	*E. coli, Salmonella,* *S. aureus, Shigella,* *L. monocytogenes*	10^2-10^4 CFU/mL	3-10 h	Jothikumar and Griffiths (57) Krascsenicsová *et al.* (58)
NASBA	Contaminated meat, fish, poultry, ready-to-eat salads, cooked dishes, water	*S. serovar Enteritidis,* *E. coli, L. monocytogenes, Bacillus, Vibrio, M. tuberculosis, Chlamydia pneumonia*	10^2-10^5 CFU/mL	1-5 h	Compton (24) Fakruddin *et al.* (32)
LAMP	Pork products, chicken, shell fish, beef, green vegetables, freshwater, eggs, raw minced meat, spiked produce samples, dairy products, honey	*Shigella, E. coli, Vibrio,* *Salmonella* spp., *S. aureus,* *S. pneumoniae, Clostridium, Yersinia*	10^2-10^4 CFU/mL	1-3 h	Saharan *et al.* (62) Song *et al.* (68) Yamazaki *et al.* (66) Wang *et al.* (64)
SDA	Raw milk products, cheese	*Mycobacterium* spp.	10^2-10^6 CFU/mL	<1 h	McHugh *et al.* (72) Walker *et al.* (71)

CFU, Colony forming units; mL, milliliter; h, hour

efficient technique for identification of several pathogens. PCR has many advantages, such as (i) simplicity, (ii) technical ease of use, (iii) time-savings, and (iv) validation and standardization in many laboratories. Nevertheless, this technique has limitations since it requires: (i) an enrichment step, (ii) extensive DNA purification, (iii) a thermal cycler and a specific thermal profile for cycling, (iv) a detection protocol for the amplicon, and (v) a qualified laboratory agent. In addition, risks of non specific amplifications usually exist (75).

Limitations in the PCR method raised demand for development of the more efficient amplification strategies of isothermal nucleic acid amplification methods. These recently developed alternative strategies, which are time-saving and cost-effective, are promising techniques for food-borne pathogen detection and identification and can be useful in laboratories where quantitative PCR equipment may be lacking. A brief summary of key properties, advantages, and drawbacks, as well as some examples of application and improvement of nucleic acid based-techniques, are given in Table 1 and 2.

In summary, recent nanotechnologies require development for transformation of potential into effective technical solutions for routine food analysis. Importantly, the technologies of these methods are not always ideal and continuous development and optimization of these novel technologies will be important for genus, species, and subspecies identification of many important pathogens implicated in severe food poisoning infections.

Disclosure The authors declare no conflict of interest.

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