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Recent and the Latest Developments in Rapid and Efficient Detection of Salmonella in Food and Water

Lan Hu and Baoguang Li*

Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Laurel, MD, USA

***Corresponding author:** Baoguang Li, Division of Molecular Biology, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Laurel, MD 20708, USA, Tel: (240) 402-3620; Fax: (301) 210-7976; E-mail: baoguang.li@fda.hhs.gov

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Abstract

Salmonella is a leading cause of foodborne diseases. Gastroenteritis caused by nontyphoid Salmonella is still a major infectious disease in the world. About 95% of Salmonella infections are caused by ingestion of contaminated food and water. Rapid, sensitive, and efficient detection and identification of Salmonella from foods and water are critical for minimizing the spread of outbreaks caused by this pathogen. These methods can be applied to track the food source of contamination or by early diagnosis of the infections in a clinical setting. Culture-based methods for detection of Salmonella are laborious and time-consuming, typically taking 5-7 days to obtain a pure culture and serovar identification. In the past few decades, molecular-based technologies have greatly shortened the time for detection and identification of bacterial pathogens from food and water, and drastically increased the specificity and sensitivity of the assays. In this review, we report an update on the development of rapid and efficient methods for the detection and identification of Salmonella in food and water, focusing on the approaches for concentration of Salmonella cells and viable cell detection, as well as the advantages and drawbacks of these methods.

Keywords: Salmonella; Pathogen detection; Molecular methods; Food; Water; Food safety; Outbreaks; Limit of detection

Abbreviations NTS: Non-Typhoid Salmonella; LOD: Limit of Detection; PCR: Polymerase Chain Reaction; qPCR: quantitative PCR; IMS: Immunomagnetic Separation; VBNC: Viable but Non-Culturable; IMS: Immunomagnetic Separation; ELISA: Enzyme-linked Immunosorbent Assay; ECL: Electrochemiluminescence; EIA: Enzyme Immunoassay; LAMP: Loop-Mediated Isothermal Amplification; ESI: Electrospray Ionization; MS: Mass Spectrometry; MALDI: Matrix-Associated Laser desorption ionization; MALDI-TOF: Matrix-associated Laser Desorption Ionization-time of Flight; AMR: Antimicrobial Resistance; PFGE: Pulse-Field Gel Electrophoresis; MLST: Multilocus Sequence Typing; WSG: Whole Genome Sequencing; SGSA: Genoserotyping Array; RT-PCR: Reverse-Transcriptase PCR; cDNA: complementary DNA; EMA: Ethidium Monoazide; PMA: Propidium Monoazide

Introduction

Salmonella, a member of the Enterobacteriaceae family, is a leading cause of foodborne diseases. Most of Salmonella isolates from human infections belong to *S. enterica* subspecies *enterica*. This genus is further divided into over 2600 Salmonella serovars (serotypes) based on the O (somatic), H (flagellar) and Vi antigens [1,2]. The most common serovars that cause human infections are *S. typhimurium* and *S. enteritidis* [3,4]. Worldwide, the incidence of non-typhoid Salmonella (*S. enterica* serovars other than Typhi or Paratyphi, NTS) is approximately 93.8 million cases, which results in about 155,000 deaths each year [5]. In the United Sates, approximately 1.4 million of salmonellosis cases are reported annually that include nearly 19 000 hospitalizations and 400 deaths [6]. The actual numbers of infections could be 10-100 times greater than the reported incidences. Gastroenteritis caused by NTS is still a major infectious disease in the world. About 95% of NTS infections are caused by ingestion of contaminated food and water [7]. Although the mortality of Salmonella gastroenteritis is lower than 1%, severe illness may occur among infants, the elderly, and the immune-compromised individuals. In sub-Saharan Africa, NTS causes invasive infections, resulting in about 20-25% cases of fatality [3]. Non-typhoid Salmonella is found in the environment and the intestinal tracts of domestic and wild animals including chickens, turkeys, ducks, geese, cattle, sheep, goats, pigs, shellfish, lizards, and turtles [8,9]. In developed countries, most human Salmonella infections occur via the ingestion of contaminated meat, egg and milk [10]. In the United Sates, Salmonella outbreaks are most commonly associated with uncooked/under cooked poultry (30%), pork (8%), beef (8%), eggs (24%), fish (2%), dairy (5%) [11]. The consumption of contaminated fresh vegetables and fruits, such as alfalfa sprouts, tomatoes, lettuce, spinach, melons, and cantaloupes is also an important source of Salmonella infections [9,12,13]. In addition, Salmonella has been isolated from a variety of dried food or processed products such as peanut butter, whole egg powder, cereals, herbs, chocolates, almonds, pecans, soy bean meal, spices and dried mushrooms [14]. Water has been considered an important source of Salmonella contamination and infection, particularly irrigation water contaminated by manure, animal faeces or sewage effluents [15]. Some Salmonella strains can persist and survive in water, soil and in the intestinal tracts of animals [8,16].

Adults with salmonellosis shed Salmonella in their faeces for about a month, while young children <5 years old of age shed 7 weeks [17,18]. Non-typhoid Salmonella can be transmitted from these temporary carriers to the other susceptible persons or from directly contract with pets such as cats, dogs, rodents, reptiles or amphibians [19,20].

Rapid and efficient detection and identification of Salmonella from food and water are critical for tracking the source of contamination and recommendations to issue a recall of contaminated food. Methods

for Salmonella detection include traditional culture-based methods, microscopic observation, immunology-based assays, nucleic acid-based assays, mass spectrometry, and biosensors. Culture-based methods for detection of Salmonella are laborious and time-consuming procedures, which often require 5-7 days for culturing and serovar identification.

In the past several decades, molecular biology technology has greatly shortened the detection time of bacterial pathogens from food and water and increased specificity and sensitivity. Rapid detection methods are capable of reducing detection time to 48 h or less. However, due to low number of the target cells in the samples and the presence of internal interfering factors such as amplification inhibitors in the food matrices, in few cases, molecular biology methods can be directly applied for detection and identification of Salmonella in food and water samples. The limit of detection (LOD) of polymerase chain reaction (PCR) and quantitative PCR (qPCR) is about 10^2 bacteria/reaction, while target cell concentration in food or water samples can often improve LOD considerably. In most cases, food and water samples have to be subjected to additional steps, such as separation and concentration, prior to being assessed with molecular detection methods. Common procedures used for separation and concentration include filtration, centrifugation, immunomagnetic separation (IMS).

In this review, we report an update on the methodologies for rapid and efficient detection and identification of Salmonella in food and water, with a focus on the methods that are developed for concentration of samples with low number of bacteria and the methods that can be used to differentiate viable bacterial cells from dead cells, as well as the advantages and drawbacks of these methods.

Specific Nature of Food and Water Samples

In most cases of salmonellosis, isolation and identification of bacterial pathogens from contaminated food and water are challenging due to numerous factors.

Complex composition of food and water samples

Most foods are composed of many different components, including proteins, polysaccharides, nucleic acids, fats and oils. The target bacteria may present in food matrix as a single cell or clumps of cells or homogenously embedded in extracellular polymeric substances (biofilms).

Interference caused by food and food ingredients or debris in bacterial isolation and detection

The target cells in some food samples may be difficult to be separated or dissolved due to food or food ingredients or debris [21,22].

Background microorganisms

Some food or water samples may contain indigenous microflora [21,22]. Most of the bacteria may be non-pathogenic microorganisms, and sometimes, Salmonella may be present in a very low number in natural microflora. The high level of DNA of non-pathogenic microorganisms may compete with the target DNA and thus affect the sensitivity and/or specificity of molecular biology methods such as PCR assays [23].

Short shelf lives of some food and food products

The detection time is critical for source tracking during an outbreak investigation. Some foods, especially fresh food products, have very short shelf lives. If the detection time to results is too long, the food products are easy to spoil, which may delay the clinical diagnosis of the Salmonella infection and affect the removal of the contaminated food and the control of the infection outspread.

Low number of the target bacterial cells in food and water

The target bacterial level in food and water is typically very low. It is reported that Salmonella can be present at approximately 1 bacterial cell per 20 g food in outbreaks such as in flour, paprika flavored crisps, tahini, ice cream and herbal tea, and 1 bacterial cell per 250 g of powder infant formula [24].

PCR inhibitors present in food and water samples

Food or water samples may contain inhibitors which may directly bind to DNA to obstruct amplification or affect the function of polymerases. These inhibitors include high concentration of collagen, myoglobin, hemoglobin, lactoferrin in meats, proteinases and calcium ions in dairy products, and polysaccharides, hemic acid, and polyphenols in fruits and vegetables [25,26].

Difficulty to recover on culture media

The target bacteria in some food samples, especially in fresh products and spices, may not grow on pre-enrichment or selective media due to the stress of the high levels of indigenous microflora or antimicrobial agents in the samples or because of cell injury [21,22]. Salmonella cells could enter a “viable but non-culturable” (VBNC) state, in which they are alive but have lost the ability to grow on classic microbiological media [27,28].

Separation and Concentration of Salmonella from Contaminated Food and Water

Isolation of Salmonella is challenging when the target bacterial number is low compared to the total bacterial number in the testing samples [29]. For most food and water samples, before detection methods can be successfully employed, separation of the target cells from the food matrix and enrichment of target cells is an essential step for Salmonella detection. Currently available PCR assays generally require concentration or pre-enrichment step to allow the target organisms to reach to 1-100 bacterial cells per 25 g of food product. This concentration or pre-enrichment steps significantly increases the overall detection time. To solve the setback, a wide range of procedures including physical, chemical and immunological separation and enrichment methods have been developed toward practical applications for rapid detection of Salmonella from contaminated food and water samples.

Sample Suspension

Food samples are usually suspended in water or culture media prior to being subjected to different analytical methods. In fact, almost all current pathogen detection methods for food and water require a suspension step and/or a pre-enrichment step. The most common solutions for sample suspension in pathogen detection are water and liquid media, such as buffered peptone water.

Sample Concentration

Once bacterial cells are released from a food matrix into suspension, the target pathogens are further separated and concentrated for detection. A variety of techniques have been developed for sample concentration including filtration, centrifugation, floatation and immunomagnetic separation (IMS) [30,31].

Filtration

Membrane filters are microporous plastic films with specific pore size ratings. The pore size for microbiological separation are 0.2, 0.45, 0.8 μM. Among these sizes, 0.2 or 0.45 μM pore size is designed for maximum recovery of Salmonella cells. The larger pore size permits faster filtration if the water sample has a high particulate burden.

Filtration is a simple and low-cost operation and has the potential to process large volumes of suspension quickly, especially for water samples [32]. However, the main disadvantage of this filtration approach is that many food suspensions cannot be easily filtered, especially large food particles or high-fat processed food products, which can clog filter membranes. The problem can be solved by incorporating additional procedures prior to detection such as using large pore size filters to remove large debris, or by pre-treating with chemicals or enzymes such as trypsin and endopeptidase to digest or degrade the food into small, soluble proteins and peptides so that they could pass through filters. However, such treatments might introduce artificial bias in detection, such as, target cells might get lost during the filtration or damaged during the enzyme digestion [33,34].

Centrifugation

Centrifugation has also been successfully applied for separation and concentration of microbial cells from food suspensions. Lower speed centrifugation can be used to remove food debris, leaving bacterial cells in the suspension, while higher speed centrifugation can be used to concentrate the organisms. The drawback for this approach is its inability to process large volumes of liquid samples.

Flotation and sedimentation

Flotation is based on buoyant density gradient centrifugation, which can separate bacterial cells from complex food particles with different densities by flotation and sedimentation, and sometimes this method can also be used to remove PCR inhibitors [35,36]. Density gradient centrifugation can rapidly and easily separate the target bacteria from food matrix.

Immunomagnetic separation (IMS)

IMS technology is a rapid, simple, and effective separation and concentration approach, and it has been widely used to separate target pathogens from food samples [31,37,38]. With the IMS method, antibodies to Salmonella are linked to paramagnetic beads allowing for specific separation of Salmonella from other microorganisms in the food or water samples. After a short incubation with the beads, the target bacteria are bound to the paramagnetic beads, and then are removed from the system using a magnet. The advantage of IMS is its rapid concentration of target cells and reduction/removal of background organisms [31]. The IMS approach can be combined with numerous detection methods such as Enzyme-linked Immunosorbent Assay (ELISA), Electrochemiluminescence (ECL) [39-41] and PCR to further improve its sensitivity. The downsides of the IMS approach are its inability to concentrate large volumes of samples, and the limitation posed by the titer and specificity of the anti-Salmonella antibodies used in the assay [37]

Combination of multiple concentration methods

Sometimes, it is necessary to combine two or more approaches, such as centrifugation, filtration and paramagnetic beads, to detect pathogen cells from a particular food or water sample. Multiple-step strategies may be suitable for complex food matrices prior to application of molecular biology techniques. For example, a combination of filtration, low and high speed centrifugation or buoyant density gradient centrifugation was reported to provide a 250-fold concentration of Salmonella in chicken samples [35].

Pre-Enrichment

After concentration, the target pathogens in many food and water samples are still below the LOD or sometimes yield false negative results due to the presence of PCR inhibitors or cell injury. In such cases, a pre-enrichment step should be employed by using a non-selective medium such as buffered peptone water or Universal Pre-Enrichment Broth [42], which can be performed by incubating at 37°C for several hours or overnight.

Most rapid detection protocols include a selective enrichment stage, which may take 4-24 h depending on the sample type, the starting concentration of Salmonella in the sample and the viability of the target organism [37,43-45]. Sometimes, a secondary selective enrichment may yield better isolation results for some food samples [44]. However, the drawbacks with enrichment include intensive labor and long incubation time; and some food samples such as fresh produce and spices, may be difficult to grow due to the high numbers of indigenous microbiota and the presence of antimicrobials found within the food commodity [21,22,46-48].

Rapid Detection and Identification of Salmonella

Rapid detection and identification methods can reduce detection time to 48 h or less. Rapid Salmonella detection utilizes several different technologies, including nucleic acid-based assays such as PCR, loop-mediated isothermal amplification (LAMP), DNA microarrays, and sequencing; immune-based methods such as Enzyme Immunoassay (EIA) and Enzyme-linked Immunosorbent Assay (ELISA); other methods such as mass spectrometry and biosensors.

Nucleic Acid-Based Detection and Identification Methods

The most common molecular detection and identification approaches include PCR, LAMP, DNA microarray, and metagenomics, as well as typing/subtyping methods.

PCR and Quantitative Real-Time PCR (qPCR)

Conventional PCR and qPCR are among predominantly rapid and sensitive detection methods of Salmonella in food and water samples.

The advantages of PCR/qPCR assays include: i) rapid time to result. PCR/qPCR may rapidly detect presumptive Salmonella directly or after the pre-enrichment stage; ii) high sensitivity and specificity [43,49-51]; and iii) the potential to amplify lower number of organisms and non-culturable bacteria.

The disadvantages of PCR assays include: i) a need for thermal cycling instrument and trained personnel; ii) the potential inhibitors in food matrices may hamper PCR amplification [52]; iii) the LOD is approximately10^2 cells/reaction for qPCR. Thus, the concentration and/or pre-enrichment steps are necessary for most food and water samples to reach the detection limit because food and water usually contain lower level of pathogens; and iv) PCR assays cannot be employed to differentiate between viable and dead bacteria. Due to amplification of DNA from both viable and dead cells within samples, PCR and qPCR assays may lead to overestimation of target cells [53] or may get false-positive results, resulting in unnecessary product recalls and economic losses [54]. Figure 1 shows the presumptive procedure of PCR/qPCR used for detection and identification of Salmonella in food and water [29].

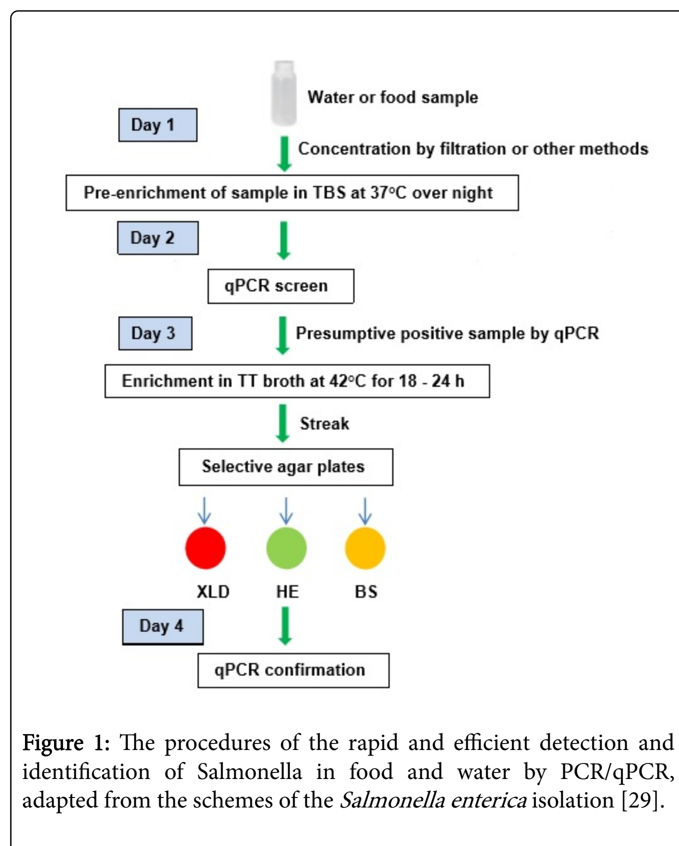

Figure 1: The procedures of the rapid and efficient detection and identification of Salmonella in food and water by PCR/qPCR, adapted from the schemes of the *Salmonella enterica* isolation [29].

Gene targets for Salmonella detection are largely virulence genes and housekeeping genes including invasion-related gene *inv*A, flagellin gene *fli*C, capsular gene *via*B, other virulence-related genes, and the 16S rRNA gene [55-57]. The *inv*A gene encodes an invasion protein, which has been proven to be Salmonella-specific [33]. It is reported that nearly 2000 Salmonella serovars contain this gene [58,59].

LAMP

LAMP is an inexpensive alternative method to PCR for the detection of bacteria in food [60]. It requires a polymerase and several sets of primers, typically 3-4 sets, which recognize a total of six or eight distinct sequences on the target DNA. This approach uses a single amplification period at a constant temperature of 60-65°C within 1 h, resulting in about10^9 copies of target DNA [60,61]. The products can be determined directly via photometry for turbidity. LAMP can be performed in a heater or water bath because it requires only one constant temperature.

LAMP is a specific, sensitive, simple, and low-cost technique, which make it a potentially valuable tool for testing foodborne pathogens in fields where standard laboratory equipment is not always readily available [62]. The limitations of LAMP are that it requires multiple sets of primers which may be difficult to design, and the inability to distinguish between viable and dead Salmonella cells form food and water samples.

DNA Microarray

DNA microarray is a high-throughput technology for simultaneous determination of the presence or absence of a large number of genes, including housekeeping genes and virulence genes [63-68]. DNA microarray has been employed to detect and identify various bacterial pathogens in food in one experiment. The 23S rRNA and 16S rRNA genes are also frequently used for detecting and identifying Salmonella [69]. Recently, a high density microarray was used to detect and identify Salmonella isolates from irrigation water [29,66].

Metagenomics

Metagenomics is a recently developed methodology. It can be used to directly analyze the microorganisms within a sample by sequencing all the genomes in the sample and comparing the genomic data to those of known microorganisms [70-75]. In addition to identify the bacteria present in the sample, this method can also be used to analyze the genetic relationship among the organisms assessed, identify putative virulence factors and explore new or rare pathogens. Metagenomics functions as pooling all technologies for detection, identification, and subtyping of Salmonella into a single assay thus it reveals microbial community directly from food samples or after pre-enrichment stage within 24 h [76,77]. The drawbacks of this technology are that metagenomics is expensive and it requires specific instruments and long data process time, etc.

Immune-Based Rapid Detection and Identification Methods

Rapid immunological detection and identification including latex agglutination, enzyme immunoassays (EIA) [78-80] ELISA [81-84] have been developed for Salmonella. There are a number of commercially available assays for Salmonella detection using anti-LPS IgM and IgG antibodies [85]. ELISA usually requires 24-48 hours to get a result, and can detect ≥ 10^5 CFU/ml of bacterial pathogen. Because ELISA requires multiple steps of reagent adding and rinsing, its specificity is reported to be inferior to PCR [86].

Mass Spectrometry (MS)

Mass spectrometry is an analytical technique, in which individual molecules of a sample can be converted to ions, and the ions are moved about and manipulated by external electric and magnetic fields for detection and identification of pathogens [87-89]. The latest types of MS include electrospray ionization (ESI), matrix-associated laser desorption ionization (MALDI), matrix-associated laser desorption ionization-time of flight (MALDI-TOF). MALDI-TOF is commonly applied for bacterial analysis, which is easy to operate and per sample cost is low except the cost of the equipment. The analysis is as fast as 10 min from colony selection to identification. However, the drawbacks

with MS are that it needs pure culture, which takes time to recover and prepare from food or water; MS is also expensive and needs highly trained personnel to run and analyze data.

One commercially available system, VITEK MS, is an automated mass spectrometry for microbial identification, which uses MALDI-TOF technology. It contains comprehensive In Vitro Diagnostic Medical Devices CE marking database for analyzing bacteria and other microorganisms.

Biosensors

Biosensors are comprised of bioreceptor molecules and transducer elements, which can detect biological species rapidly and quantitatively. Biosensors have been widely used for pathogen detection at low cost. Electrochemical immunosensors employ antibodies as bioreceptors to recognize pathogens. Almost all types of immunosensors are applicable for Salmonella detection. Immunosensors allow "real-time" detection and analysis of Salmonella in food samples.

Phage-based magnetoelastic (ME) biosensors have been designed to a real-time, wireless, direct detection method for Salmonella, in which a genetically engineered phage is served as a biomolecular-recognition element [90]. When pathogens are bound to the coated phage, the increase in the biosensor mass causes a decrease in its resonant frequency. ME biosensors are simple, fast and cost-effective detection methods.

Serotyping and Genotyping

A variety of Salmonella typing and subtyping methods have been developed. Phenotypic methods include serotyping, phage typing, and antimicrobial resistance (AMR) typing, whereas molecular typing methods include plasmid profiling, pulse-field gel electrophoresis (PFGE), ribotyping, multilocus sequence typing (MLST), and whole genome sequencing (WGS). PFGE is recognized as a gold standard for Salmonella subtyping and for investigation of Salmonella outbreaks. The drawbacks of PFGE and MLST are that the methods require pure cultures and it takes 2-3 days to get results. WGS provides the greatest resolution for microbial subtyping to examine the bacterial evolution and to identify the source and transmission pathways of Salmonella. WGS is becoming popular as the costs continue to drop with the improvement of the next generation sequencing technique [91,92].

Multiplex PCR based on genes encoding for O and H antigens or genomic markers is employed to molecular serotyping. The technique has been shown to be a powerful and cost-effective tool for Salmonella typing and can get results in less than a day [93]. Salmonella genoserotyping array (SGSA) is a microarray-based method using serovar-specific genomic markers for rapidly typing.

Detection of Viable Bacteria in Food and Water

Molecular biology technologies, such as PCR assays, amplify DNA from both viable cells and dead cells as well as from extracellular DNA within samples and may overestimate viable bacteria numbers [53]. The overestimation may lead to unnecessary product recalls and economic losses [54]. Several methods have been developed for detection of viable foodborne pathogens, including reverse-transcriptase PCR (RT-PCR) [94], qPCR assays with biological dyes [33] and commercially available dead/live bacteria viability test kits (Live/Dead® BacLight™ Bacterial Viability Kit, Molecular Probes Inc., Eugene, OR).

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Reverse transcription polymerase chain reaction (RT-PCR), is a technique for detection and quantitation of mRNA, in which RNA is converted into a complementary DNA (cDNA) and then the cDNA is used as a template for amplification. Due to the short half-life of bacterial mRNA (0.5-50 min) [95], bacterial mRNA degrades rapidly than DNA after cell death. RT-PCR is considered as an acceptable cell viability indicator of bacterial cells [94,96]. RT-PCR, quantitative RT-PCR (qRT-PCR) and real-time RT-PCR (rt-RT-PCR) have shown great potential in detecting viable Salmonella cells [97-100].

The disadvantages of the assay include: i) the presence of possible contamination with genomic DNA, which often leads to false-positive results; and ii) some RNA molecules may persist in cells in a detectable form for an extended time period (about 1 h) after loss of cell viability in some specific conditions, which may lead to false positive results [101].

EMA/PMA-PCR

One approach used to differentiate viable and dead cells is pre-treating the sample with biologic dyes such as ethidium monoazide (EMA) or a derivation of EMA, propidium monoazide (PMA) before DNA extraction [102]. EMA and PMA are weak fluorescent dyes and can bind to genomic DNA, especially dsDNA with high affinity. PMA is more effective than EMA in differentiation of viable cells from dead cells [103]. Because the dyes do not permeate intact cell membranes of live cells, they can only intercalate the exposed DNA from dead cells and thus prevent amplification of the modified DNA from dead cells [104].

A PMA-qPCR assay was developed that targeted the Salmonella *inv*A gene in conjunction with PMA-qPCR treatment for detecting viable Salmonella in food [33]. The length of the PCR amplicons is a key factor to successfully detect viable foodborne pathogens and an amplicon of 130 bp is the most optimal length for successful PMA treatment [33]. The concentration of the dyes, the numbers of tested bacteria, ratios of viable and dead cell and the sample types are main factors that affect the efficiency of PMA-qPCR. If the factors are carefully considered, the EMA/PMA-qPCR assay is a sensitive and reliable method for detecting viable Salmonella in food and water samples.

The disadvantages of EMA/PMA-PCR may include that the degree of cell membrane permeability may vary. High numbers of dead cells in the presence of much lower numbers of viable cells may interfere with the detection of outcomes [33]. The false-positive enumerations of viable cells will occur if there are 100-fold higher numbers of dead cells present in the sample than live cells [105]; PMA-qPCR could not provide accurate estimates of bacterial viability in environments where bacteria growth and survival are limited.

EMA/PMA-LAMP

EMA and PMA have been reported to combine with LAMP for detection of viable cells of Salmonella [106-109]. The concentration of EMA used in the assay is critical in affecting DNA amplification of

viable cells. PMA is found to be less effective in differentiating between low number of viable and heat-killed cells of Salmonella [106].

Conclusion

Gastroenteritis caused by Salmonella is still a major concern for food safety and public health agencies. Ingestion of contaminated food and water is the largest cause of salmonellosis. Rapid and efficient detection techniques are critical for prevention and control of Salmonella infection, and can greatly improve food safety by identifying the contaminated foods. However, few food and water samples can be directly applied for these detection methods due to the particular nature of these samples, such as the pathogen level of sample, types of food matrices. Thus, concentration and pre-enrichment of food and water samples are often the necessary steps for increasing Salmonella cells to detectable level prior to employing various molecular detection methods.

Detection of the viability of bacterial pathogens is important for avoiding false-positive results and unnecessary production cost. RT-PCR and qPCR with EMA or PMA treatment are practicable approaches to differentiate viable cells from dead cells. LAMP with EMA pre-treatment is a promising, cost-effective approach for field investigation and cutting down medical costs.

Taking all this into account, choosing appropriate concentration/pre-enrichment approaches and continuing to develop more sensitive, more specific and more efficient molecular methodologies are critical for detection of Salmonella in food and water and protection of our food chain.

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