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Advanced Techniques in Detecting Food Borne Pathogens and Toxins

G. Aswani*, S. Anu Rajan, N. Chithra and V.I. Soumya

Department of Agricultural Microbiology, College of Agriculture, Vellayani Pin 695522

*Corresponding Author: anu7172@gmail.com

Abstract

Detecting foodborne pathogens and toxins is critical for safeguarding public health against outbreaks of foodborne illnesses caused by bacteria, viruses, parasites, and toxins in contaminated food. Advanced detection methods have significantly improved accuracy and speed across various stages of food production and distribution. Challenges include the complexity of food matrices and the need for sensitive detection despite low pathogen concentrations. Techniques such as PCR, ELISA, and immunomagnetic separation enhance sensitivity, while nanotechnology and biosensors offer rapid and specific detection capabilities. Integration of these technologies with traditional culture methods and emerging high-throughput sequencing promises further advancements in food safety. Continued innovation is essential to develop real-time, comprehensive detection systems that minimize risks associated with foodborne pathogens and toxins globally.

Introduction

Each year, millions of people worldwide suffer from foodborne diseases caused by bacteria, viruses, parasites, and toxins present in contaminated food and beverages. These pathogens and toxins can lead to various symptoms, ranging from minor digestive issues to serious illness and, in vulnerable individuals, even death. Effective detection methods are essential for identifying and mitigating these risks before contaminated food reaches consumers. Advances in technology have significantly improved our ability to detect foodborne pathogens and toxins quickly, accurately, and at various stages of food production and distribution. The importance of food safety, the challenges involved in detecting pathogens and toxins, and the role of technology in enhancing our capabilities in this critical area. By understanding and implementing robust detection methods, stakeholders in the food industry can collaborate to minimize the occurrence and impact of foodborne illnesses, thereby ensuring safer food for everyone.

Challenges associated with food analysis

Identifying microbial pathogens and toxins in foods poses several difficulties linked to food analysis. Food products are diverse, ranging from complex matrices to nonviable cells, which complicates detection methods. Detecting microbial pathogens and toxins in foods presents several challenges associated with food analysis. Additionally, concentration and extraction techniques are employed to enhance detection sensitivity; however, they often concentrate inhibitory components, affecting assay reliability. Poor recovery rates in extraction and purification processes further diminish assay sensitivity and efficiency. These inherent complexities underscore the need for innovative approaches and technologies to overcome these challenges and ensure effective food safety measures.

Preparing the sample

An extensive examination for pathogens and toxins in foods consists of three key stages: sampling, sample preparation, and detection assays. Although the assay itself may be quick, spanning minutes to hours, the entire analysis process can extend over several days. Pathogens in food are typically found in low concentrations, requiring the processing of large sample volumes for reliable detection. Different methods are used to concentrate and separate food matrices, such as physical techniques like centrifugation and filtration, chemical methods involving substances like metal hydroxides, resins, and lectins, and immunomagnetic separation (IMS) which uses magnetic beads coated with antibodies specific to pathogens.

Modern pathogen testing methods frequently include a single or dual-stage broth enrichment process to increase the concentration of the target pathogen to detectable levels. Following enrichment, rapid screening tests like enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are utilized for timely determination of negative samples. Combining these methods offers advantages in achieving necessary test sensitivity and specificity, as well as facilitating rapid detection.

Conventional Culture Methods

Conventional techniques for microbial analysis of foods, widely regarded as the benchmark for detection, typically entail multiple incubation phases, including pre-enrichment, selective enrichment, and plating on differential and selective agars. Subsequent confirmation and characterization utilize biochemical,

serological, or molecular tests. Central to these techniques is the employment of selective and differential culture media: selective media foster the growth of desired pathogens while hindering other organisms that might hinder detection. Differential media, on the other hand, rely on distinct biochemical characteristics exhibited by target organisms, often evidenced by colour changes within the growth medium.

Recent advancements include the development of enhanced media formulations for selective enrichment and identification. These innovations aim to improve the sensitivity and specificity of pathogen detection in food, ensuring more reliable and accurate results.

Rapid Methods

Advancements in detecting microbial pathogens and toxins in foods have progressed significantly with rapid methods, which include convenience-based, antibody-based, and nucleic acid-based assays. Convenience-based assays like hydratable media gel cards (e.g., Petri film) and ATP testing devices offer rapid feedback on food sanitation conditions but do not directly measure microbial contamination levels. Specialized substrate media such as chromogenic agar facilitate the identification of particular pathogens like *E. coli* O157 and *Listeria monocytogenes*. Miniaturized biochemical assays such as API systems are extensively utilized for pathogen identification.

Antibody-based assays utilize antibodies to directly identify pathogens or aid in their detection through immunomagnetic separation (IMS), which concentrates pathogens before detection by techniques like PCR. Nucleic acid-based assays involve hybridization with probes or amplification via PCR and related technologies. Real-time PCR offers rapid amplification and simultaneous detection, crucial for quantifying pathogens in foods. Techniques such as loop-mediated isothermal amplification (LAMP), which operate at a constant temperature, have been developed for detecting bacteria and viruses in food samples.

Despite their speed, rapid methods are typically used as screening tools, requiring confirmation of positive results through traditional culture-based methods. Additionally, enrichment steps are often necessary to achieve the sensitivity required for food testing, thereby extending the total analysis time. Techniques incorporating propidium monoazide or ethidium bromide monoazide show promise in differentiating live from dead cells in molecular assays, enhancing accuracy in complex food samples.

Nanotechnology

Nanotechnology has emerged as a revolutionary method for detecting pathogens and toxins in food, providing advanced solutions to improve both sensitivity and speed. A notable example involves dye-doped silica nanoparticles, where dye molecules are integrated into nanostructures and conjugated with antibodies specific to *E. coli* O157. This method enables the detection of as few as 1 CFU/g of bacteria in ground beef within just 20 minutes. Furthermore, nanotechnology has been successfully integrated with microfluidic systems, such as micro-PCR, capable of completing 40 cycles of PCR in less than 6 minutes, thereby significantly reducing overall analysis time.

Despite these advancements, challenges persist, particularly regarding the potential clogging of microfluidic systems by food particles. Nevertheless, the integration of nanotechnology with molecular biology techniques holds immense promise for revolutionizing pathogen and toxin detection in food safety, offering rapid, sensitive, and efficient methods that could transform current practices in food industry quality control and public health protection.

Advanced Technological Application

State-of-the-art technology applications for identifying pathogens and toxins in food. The first example combines PCR with an oligonucleotide-labelled nanoparticle detection system (Luminex, Austin, TX), employed by the Centres for Disease Control and Prevention for molecular serotyping of *Salmonella.* This method targets specific sequences of genes coding for *Salmonella* O antigen groups and H antigen phases, followed by multiplex PCR and detection via fluorescence-labelled bead sets on a Bio-Plex platform. This approach enables high-throughput, multiplexed detection of *Salmonella* serogroups and H antigen types.

The second application utilizes transcription-mediated amplification assay (TMA), developed by Gen-Probe (San Diego, CA), for rapid detection of *Listeria, Salmonella*, and *Campylobacter* genera. This method involves capturing target ribosomal RNA from food samples using poly-A-linked probes and magnetic particles, followed by isothermal amplification with real-time TMA using fluorescence-labelled molecular beacons. The TMA process, operating at 42°C for 75 minutes, achieves sensitive

and specific amplification of RNA, suitable for detecting pathogens in various food matrices.

The third application involves highly sensitive detection of biological toxins using a liposome-PCR assay. In this assay, toxin-specific antibodies immobilized in microtiter plate wells capture target toxins, which are then bound by liposomes containing reporter DNA. Upon rupturing the liposomes, real-time PCR quantifies the released reporter DNA, providing a quantitative measure of bound toxins. This assay format, resembling sandwich ELISA, demonstrates higher sensitivity compared to existing detection methods for toxins like *cholera* and *botulinum.*

These advanced technologies exemplify significant advancements in food safety testing, offering rapid, sensitive, and specific detection methods crucial for enhancing public health protection and ensuring the safety of food supplies.

Immunomagnetic separation

Immunomagnetic separation (IMS) (Mandal *et al*,2011) is a powerful assay used to isolate and identify pathogens from food samples by employing antibodies bound to functionalized magnetic beads. This method is known for its rapidity and efficiency, making it particularly suitable for handling large quantities of samples. In IMS, antibodies are initially conjugated with magnetic beads and incubated with the sample at refrigerated temperatures. Following incubation, any unbound antibodies are removed through washing steps. Subsequently, antigen-coated magnetic beads specific to the pathogen of interest are introduced into a semi-liquid food sample containing the pathogen's antigen (either the whole cell or its toxin). This mixture undergoes further incubation to facilitate the reaction between the antigen and the antibody-coated beads. IMS offers several advantages in pathogen detection, including its ability to concentrate and isolate target pathogens effectively from complex food matrices. By leveraging magnetic properties, IMS simplifies sample handling and enhances the sensitivity of subsequent detection assays. These characteristics underscore IMS as a valuable tool in food safety, contributing to the swift and reliable identification of pathogens to mitigate risks associated with foodborne illnesses.

Biosensors for Pathogen Detection:

Biosensors are emerging as rapid detection technologies compared to traditional methods like PCR, immunology, culture methods, and gel electrophoresis. They typically consist of Biologically active molecules (probes or antibodies), Transducer to convert the biological signal into an electrical signal, Data output system for interpretation.

ATP Biosensor

Uses ATP bioluminescence to detect microorganism biomass. ATP reacts with Luciferin/Luciferase to produce bioluminescence. Results are measured in Relative Light Units (RLU) and are proportional to microbial biomass. Provides results in less than 5 minutes.

Optical Biosensors

Offer specificity, cost-effectiveness, and rapid detection. Utilize techniques like light absorbance, luminescence, fluorescence, and more. Changes in optical properties upon analyte binding are detected. Common types include fiber optic biosensors which use light propagation through the core of an optical fibre.

Piezoelectric Biosensors:

Detect microbial pathogens directly. Principle involves coating piezoelectric sensors with selective binding substances (e.g., antibodies). Binding of target nucleic acids causes a mass increase and a decrease in resonance frequency of the sensor. Quartz crystal microbalance (QCM) biosensors are commonly used for label-free detection.

Each biosensor type has its advantages, such as the rapid detection capability of ATP biosensors, the specificity and cost-effectiveness of optical biosensors, and the direct detection capability of piezoelectric biosensors. These technologies collectively offer faster and more specific pathogen detection compared to traditional methods, making them valuable in various applications, including food safety and clinical diagnostics.

Gene sequencing

Sanger Sequencing (First-Generation Sequencing) (Segerman, 2020; Kaprou *et al*., 2021) Involves cloning DNA fragments into plasmid vectors, which are then sequenced using chain-termination method. Commonly used in small-scale sequencing tasks, such as sequencing bacterial genomes and plasmids. Known for its accuracy, precision, and ability to target specific sequences

Next-Generation Sequencing (NGS)

Second-Generation Sequencing (Synthesis Sequencing)

High throughput, reduced cost per base, and faster sequencing compared to Sanger. Examples: Roche's 454, Illumina's Solexa, Hiseq, ABI's SOLiD.

Principle: Relies on PCR amplification of DNA fragments attached to a solid surface, followed by sequencing-by-synthesis.

Third-Generation Sequencing (Single-Molecule Sequencing)

Longer read lengths, reduced bias from PCR amplification, and potentially lower sequencing error rates. Sequences DNA molecules directly as single molecules without the need for PCR amplification. Examples: Helicos Biosciences' HeliScope, Pacific Biosciences' PacBio RS, Oxford Nanopore Technologies' GridION and MinION.

Applications and Advancements:

High-Throughput Sequencing (NGS)

Revolutionized molecular biology by enabling simultaneous sequencing of millions of DNA molecules, essential for large-scale genomic studies, metagenomics, and transcriptomics. Specific Examples: Microbial Community Analysis: Used in studies like analysing bacterial colonies in butter, detecting uncultured microbes in food samples, and characterizing STEC in Chile. Serotype Prediction: Demonstrated accurate serotype prediction in Salmonella using whole-genome sequencing data from multiplex ONT sequencing.

Future perspectives

An optimal system for detecting pathogens and toxins in foods should demonstrate exceptional specificity and sensitivity, rapid response times, scalability for mass production, simplified sample preparation, minimal disturbance to samples, and continuous data analysis. Despite advancements in automated and high-throughput sample processing and testing, there is still a noticeable lack of truly seamless 'real-time' procedures that encompass sampling through to obtaining results. The culture enrichment step, which currently consumes a significant portion of testing time, persists because of its benefits, particularly its ability to enhance sensitivity. To meet the goal of real-time testing, innovations such as enhanced media formulations for rapid enrichment and alternative concentration technologies are required.

Creating a standardized sample preparation technique is essential for progressing the future of pathogen and toxin detection methods in food. This requires interdisciplinary collaboration among fields such as chemistry, engineering, molecular biology, microbiology (clinical, environmental, and food microbiology), and food science.

Furthermore, there is growing importance placed on features like quantification and multiplex detection in microbial testing technologies adapted from chemical and physical testing domains, such as mass spectrometry (MS) and optical scanning technologies. Integrating these technologies with expertise from various scientific disciplines holds the potential for significant advancements in pathogen and toxin detection methodologies for food safety.

Conclusion

Advancements in detecting foodborne pathogens and toxins have significantly enhanced our ability to ensure food safety and protect public health. Technologies such as PCR, ELISA, IMS, and biosensors offer rapid, sensitive, and specific detection capabilities crucial for identifying contaminants early in the food supply chain. Despite challenges in sample preparation and the need for ongoing innovation, these methods represent critical strides towards more effective food safety measures. Integrating nanotechnology and advanced sequencing techniques further promises to revolutionize pathogen detection, enabling quicker responses and more comprehensive monitoring of foodborne risks. Continued collaboration and research across disciplines will be essential for developing future-proof solutions that mitigate the impact of foodborne illnesses globally.

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