DETECTION METHODS IN FOOD BIOTECHNOLOGY

Abstract

Food borne pathogens cause numerous diseases globally, particularly in developing countries, causing significant economic impact. Early detection is crucial for containment. Detection methods have evolved from culture-based methods to immunological and molecular biology-based approaches. The goal is to find rapid, sensitive, specific, and cost-effective methods, including microbe culturing and biosensor technology. Food safety is crucial for livelihoods and millions of people are affected by issues. Collaboration between government agencies, food processing businesses, and private consumer organizations is essential for improving food inspections. Bioassay technology offers benefits like efficiency, precision, and simplicity, making traditional procedures imprecise. Advancements in science and technology encourage the implementation of biotechnology, providing high sensitivity and specificity in monitoring food safety.

Keywords: Collaboration between government agencies, food processing businesses, and private consumer organizations is essential for improving food inspections.

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I. INTRODUCTION

The preservation of the food supply's safety depends heavily on biotechnology. Both food producers and consumers will greatly benefit from the establishment of trustworthy procedures to guarantee the presence of transgene. Better tracking techniques will boost public trust in food biotechnology. The development of sensitive, dependable, quick, and affordable technologies for the detection of dangerous pathogenic organisms in food supply and the infectious agent like for mad cow disease also make use of contemporary biotechnology tools.

Contaminated food causes potential health risks and thus a major concern globally. Globally, by ingesting food contaminated with pathogenic micro-organism and toxic chemicals 600 million people falling ill and 420,000 deaths are occurring each year [1]. Thus detecting contaminants in food is crucial for food safety. The detection methods should be rapid and accurate to ensure food safety. Due to the lack of sensitivity, time consuming and accuracy in traditional methods of food pathogen detection like biochemical detection and microbial isolation new advanced techniques with high specificity, accuracy, ease to use methods are being evolving. Other than traditional methods , advanced detection techniques including nucleic acid based methods like conventional PCR, Real time PCR, digital PCR, nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), biosensors, microbial based biosensors, surface plasmon resonance (SPR) technique ,and Cas-based nucleic acid detection systems (CRISPR) methods. Compared to traditional methods these new advanced techniques detect food borne pathogens with high sensitivity, rapid turnaround time and specificity. Hence, these methods are enhancing our capacity and knowledge in detecting food borne contamination and facilitating the consumers in ensuring to take safe food, making these techniques crucial and urgent need in food market [2].

II. TRANSGENE DETECTION

Food biotechnology success requires ongoing product development and effective commercialization through market acceptance. The creation of trustworthy ways of detecting the transgene in human food items is must for consumer's satisfaction and for their endorsement of the use of transgenic food products [3]. But there is also a higher risk of transgenic product contamination in non-transgenic products as the number of GMOs (genetically modified organisms) allowed for production and commercialization rises. One such well reported incident occurred when Safeway and Taco Bell in October 2000 voluntarily recalled a maize product after discovering traces of genetically modified corn in them. For these and many more reasons, the effectiveness and acceptance of GMOs in the future will depend on accurate transgenic product identification techniques. Real-time qPCR (RT PCR) is the most potent, accessible, and economical transgenic product detection technology currently in use [4]. Choosing the unique gene sequence to amplify in PCR is the

major issue to implement in this detection technique. The major components used to detect GMOs today are signature sequences, such promoter sequences and antibiotic resistance gene markers however, they are not optimal because the same signature sequences can be found in other types of GMO. Additionally, there is an untested worry that these antibiotic resistance gene markers and signature sequences, may result in negative effects on human health and the environment. The European Union, which has strict regulations on GMOs, outlawed the use of antibiotic resistance gene to use as markers for cloning and transformation of particular genes from year 2004 in order to allay this worry. A threshold level of 1% for the presence of transgenic product was also established for the mandatory labeling of GMO foods by the European Union, which prompted more aggressive research on highly specialized, exact, and sensitive techniques for detecting and quantifying transgenic products in food products.

A revolutionary concept for the global identification of GMOs was created by researchers for the German company Icon Genetics [5]. The development of a standardized process for the addition of non transcribed DNA-based technical data to the transgene prior to its insertion into the organism's genome was suggested. According to Marillonet *et al*., 2003 [5], this coding would be based on triplets codons , just as amino acid codons, and each triplet codon should encode for one of the 26 english alphabets , and an Arabic numeral from 0 to 9, and one space character, for a total of 37 characters. The scientists were able to incorporate into these characteristics biologically inert, non-genetic coding sequences that correspond to distinctive data like the brand name of the business, the date and location of manufacture, the model of the product, and the serial number. Cloning will take place between conserved sequences that have primer-binding domains and the variable region that contains the information. Only PCR and fragment sequencing are required to read the DNA's encoded information.

III. FOOD PATHOGEN DETECTION

The toxin producing bacteria like *Vibrio, Salmonella, Listeria,* and *lethal strain of E.coli (O157:H7)* which secretes toxins called shigha toxin contaminate food and cause food poisoning. The shigha toxins are encoded by genes stx1 and stx 2. These shigha toxins (stx1 and stx 2) when appeared in blood stream it can induce damage to other organs like kidney and inner lining of large intestine leading to severe diarrhea and dehydration [6]. Primarily this lethal strain of *E.coil* is prevelanet in North American cattles' intestine. These lethal strains are spreads in community through unpasteurized milk, ground beef and roast beef. The O157:H7 strain is primarily prevalent in the intestines of healthy cattle in North America. According to the Centre for Disease Control (CDC), ground beef, unpasteurized milk, and roast beef are the main sources of food borne transmission for Shiga toxin-producing *E. coli* (STEC), such as O157:H7, which is estimated to cause 73,480 illnesses and 61 fatalities annually in the United States alone [7].

Greater effort has been made to develop quick and accurate procedures for food contamination identification. Because PCR provides quick, precise, and extremely sensitive results—in contrast to traditional methods— PCR based methods become more preferable techniques for food pathogen detection. Shiga toxins (stx) [8], intimin [9], enterohemorrhagic *E. coli* hemolysin [10], and -glucuronidase (EC 3.2.1.31) [11] are detected through PCR amplification. However in conventional PCR following gel electrophoresis the quantity of sample analyzed is limited. To overcome this limitation scientist from Centre for Food Safety

and Applied Nutrition, Food and Drug Administration, Washington, D.C., and the Department of Nutrition and Food Science, University of Maryland, College Park, Maryland, developed an assay for quick and large scale detection. This method involves analysis of PCR product using ELISA (Enzyme linked immunosorbent assay). This method includes labeling of digoxigenin- dUTP and a biotin-labeled primer for the stx1 and stx2 genes. This PCR-ELISA method can be used to detect E. coli O157:H7 and other STEC in food in large scale level. After the PCR amplification the PCR products were added to microtiter wells already coated with streptavidin. In this procedure, and the ELISA was used to identify them using an anti-DIG-peroxidase conjugate. Other immunoassays like flow injection immunoassay, enzyme-linked fluorescent assay (ELFA), and few other serological assays, are known for quantification of the target organisms [13], but due to lack of sensitivity and selectivity, limits its widespread use.

In various fields like food, agriculture, medical and pharmaceuticals other label free detection methods such as spectroscopic methods using signatures of absorption *via* electromagnetic radiation, nuclear magnetic resonance, fluorescence, laser light and mass spectroscopy are being employed [14, 15, 16, 17]. As an alternative, bio-recognition technique is an alternative emerging technique to ensure food safety [18]. The development of new bio-recognition ligands is providing good opportunities for designing and development of sensitive methods for detecting microorganisms in recent years. Another alternative, Biosensors are providing rapid microbial detection techniques for the detection of bacteria in food [19]. Biosensors consist of sensing elements which is made up of bio macromolecules and a transducer that transfer signal to a visual recordable signal. Biosensors are user friendly, rapid, sensitive and specific for the detection on pathogens or toxins [19]. Another methods like LAMP and NASBA are sensitive, specific, and cost-efficient, making them useful in low resource settings [19].

IV. SURFACE PLASMON RESONANCE (SPR)

The SPR biosensing technology enables for real-time monitoring in done at the interface of a dielectric/ transparent medium and a thin gold film of chemical and biochemical interactions. The evanescent wave phenomenon is used in this optical approach to monitor change in refractive index(RI) near to the sensor surface. Any alteration at the metal-dielectric contact has a large impact on the angular position. Many real-time monitoring SPR approaches rely on the prism-based Kretschmann configuration, which employs the metal-side excitation of a surface-bound electromagnetic wave. The occurrence of antigen-antibody binding events may be tracked by observing the movement of the angular minimum towards higher angles or the variation in reflectance at a constant angle.

Other than normal SPR, optical SPR biosensor is also used. This method allowed multiple and simultaneously analysis of analytes. The current SPR technology is integrated in detection system to increase feed, food, and environmental safety [20, 21].

V. BIOSENSORS

Electrochemical biosensors transform the interaction between an analyte and biorecognition agent to electrical signals. The electrical signals are proportional to the concentration of the detecting analyte. Electrochemical biosensors can be of different types depending on the parameter being measured, they can be, impedimetric, potentiometric or

amperometric. Xu, Wang, and Li (2016) developed an electrochemical biosensor with a limit of detection (LOD) of 102 CFU ml1 for detecting E. coli O157:H7 in food, water, and environmental materials [22]. These biosensors, however, have limitations, such as the possibility of enzyme-substrate reactions and redox hindrances. Other biorecognition components, such as non-functionalized gold nanoparticles and nucleic acids, might be employed. Furthermore, the homogeneous distribution of microbial pathogens in food, water, and environmental samples makes electrochemical approaches challenging to apply, particularly in the absence of sample preparations.

Recently, based on smartphones some modern efficient analytical method involving electrochemical biosensor and optical aptasensor have been developed for detection of food pathogen contamination. This techniques involve several bioreceptors like aptamers, enzymes, antibodies, microorganisms and cells have been integrated with smartphone-based biosensors [23].

VI. MICROBIAL BIOSENSORS

Microbial biosensors are analytical devices that combine microorganisms with a transducer to detect targets in real time. Microbial bio-sensors involve coating of antimicrobial substances and antimicrobial delivery system. These approaches include cost effective and stable bio based receptors of antimicrobial peptides, bacteriophages , DNAzymes, and engineered liposomes. Numerous delivery systems of antimicrobial substances are developed using cell-based carriers, microbubbles and lipid colloidal particles. They are more favourable than enzyme biosensors due to their complexity and expense. Three types of microbial biosensors available which are: potentiometric, amperometric and conductometric. In amperometric biosensors the current generated by oxidation and reduction reaction at electrode surfaces are measured to detect BOD (biological oxygen demand) in chemicals and industrial waste. In potentiometric biosensors employ an ion-selective electrode and/or a gas-sensing electrode covered with an immobilised layer of microbes to detect, penicillin, organophosphates, tryptophan, trichloroethylene, urea, Sucrose and ethanol. A caffeine based biosensor was created by immobilizing Pseudomonas alcaligenes MTCC 5264 in a whole cell biosensor. Alltogether, these newely evolved techniques other than traditional methods can reduce microbial contamination risks and enhance the detection of microbes in situ [24].

VII. AMINOACIDS BIOSENSORS

Approaches using (poly) amino acids probes are used for real time sensing of contaminations in food. Till now probes of numerous (poly) amino acids like poly(alanine), poly(leucine), poly(cysteine), poly(tyrosine), poly(histidine), poly(arginine), poly(lysine), poly(tryptophan), poly(glutamic) and poly(glycine), were used to detect food contamination [25].

VIII. NANOMATERIALS BASED CRISPER/CAS DETECTION SSYSTEM FOR FOOD PATHOGEN

Recently, many of CRISPR/Cas-assisted bio based sensors have been described for food safety detection. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a technique with sequence specific nucleic acid targeting capability for nucleic acid detection. This technique comes up with high sensitivity, programmability, and biocompatibility with single base resolution.

Nano-biosensors are in wide use in fields of food safety. The application of nanomaterials in biosensors attracted scientists to developed CRISPR based biosensors involving various nanomaterials to detect contaminants like food borne pathogenic viruses, GMOs, food borne pathogenic bacteria, food adulteration, toxins, pesticide residues, and antibiotic residues etc. in food get better detection success. Nanoparticles like Graphene, Quantum dots (QDs) and metal nanoparticles incorporated with CRISPR detection system to improve analytical performance. A nanomaterial has high specific surface area and binding sites which have greatly facilitated the development of CRISPR/Cas-assisted detection system [26].

IX. CONCLUSION

Conventional methods for detecting food borne pathogens are selective but time-consuming and laborious. Rapid detection methods have emerged to overcome these limitations. Nucleic acid-based methods like PCR, mPCR, qPCR, and DNA microarray have high sensitivity but require trained personnel. Biosensors-based methods have emerged for food borne pathogen detection due to their rapidity, cost-effectiveness, and ease of operation. These methods don't require trained personnel and can detect food borne pathogens. Numerous Immunological-based methods like ELISA, PCR-ELISA and lateral flow immunoassay are also used, but need improvement in food matrix detection. Combining rapid methods is also possible for more effective and accurate detection.

REFERENCES

[1] Lee, H., & Yoon, Y. (2021). Etiological agents implicated in foodborne illness worldwide. Food science of animal resources, 41(1), 1.
[2] Hajikhani, M., Zhang, Y., Gao, X., & Lin, M. (2023). Advances in CRISPR-based SERS detection of food contaminants: A review. Trends in Food Science & Technology.
[3] James, C. (2001). Global review of commercialized transgenic crops: 2000.
[4] Higuchi, R., Dollinger, G., Walsh, P. S., & Griffith, R. (1992). Simultaneous amplification and detection of specific DNA sequences. Bio/technology, 10(4), 413-417.
[5] Marillonnet, S., Klimyuk, V., & Gleba, Y. (2003). Encoding technical information in GM organisms. Nature biotechnology, 21(3), 224-226.
[6] Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., ... & Cohen, M. L. (1983). Hemorrhagic colitis associated with a rare Escherichia coli serotype. New England journal of medicine, 308(12), 681-685.
[7] Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., ... & Tauxe, R. V. (1999). Food-related illness and death in the United States. Emerging infectious diseases, 5(5), 607.
[8] Brian, M. J., Frosolono, M., Murray, B. E., Miranda, A., Lopez, E. L., Gomez, H. F., & Cleary, T. G. (1992). Polymerase chain reaction for diagnosis of enterohemorrhagic Escherichia coli infection and hemolytic-uremic syndrome. Journal of Clinical Microbiology, 30(7), 1801-1806.
[9] Gannon, V. P., Rashed, M., King, R. K., & Thomas, E. J. (1993). Detection and characterization of the eae gene of Shiga-like toxin-producing Escherichia coli using polymerase chain reaction. Journal of Clinical Microbiology, 31(5), 1268-1274.
[10] Ge, B., Larkin, C., Ahn, S., Jolley, M., Nasir, M., Meng, J., & Hall, R. H. (2002). Identification of Escherichia coli O157: H7 and other enterohemorrhagic serotypes by EHEC-hly A targeting, strand displacement amplification, and fluorescence polarization. Molecular and Cellular Probes, 16(2), 85-92.

[11] Feng, P. (1993). Identification of Escherichia coli serotype O157: H7 by DNA probe specific for an allele of uid A gene. Molecular and cellular probes, 7(2), 151-154.
[12] Ge, B., Zhao, S., Hall, R., & Meng, J. (2002). A PCR–ELISA for detecting Shiga toxin-producing Escherichia coli. Microbes and infection, 4(3), 285-290.
[13] Alahi, M. E. E., & Mukhopadhyay, S. C. (2017). Detection methodologies for pathogen and toxins: A review. Sensors, 17(8), 1885.
[14] Pan, Y., Sun, D. W., & Han, Z. (2017). Applications of electromagnetic fields for nonthermal inactivation of microorganisms in foods: An overview. Trends in Food Science & Technology, 64, 13-22.
[15] Xie, X., Pu, H., & Sun, D. W. (2018). Recent advances in nanofabrication techniques for SERS substrates and their applications in food safety analysis. Critical reviews in food science and nutrition, 58(16), 2800-2813.
[16] Yaseen, T., Sun, D. W., & Cheng, J. H. (2017). Raman imaging for food quality and safety evaluation: Fundamentals and applications. Trends in Food Science & Technology, 62, 177-189.
[17] Hameed, S., Xie, L., & Ying, Y. (2018). Conventional and emerging detection techniques for pathogenic bacteria in food science: A review. Trends in Food Science & Technology, 81, 61-73.
[18] Jayan, H., Pu, H., & Sun, D. W. (2020). Recent development in rapid detection techniques for microorganism activities in food matrices using bio-recognition: A review. Trends in Food Science & Technology, 95, 233-246.
[19] Shahdordizadeh, M., Taghdisi, S. M., Ansari, N., Langroodi, F. A., Abnous, K., & Ramezani, M. (2017). Aptamer based biosensors for detection of Staphylococcus aureus. Sensors and Actuators B: Chemical, 241, 619-635.
[20] Waswa, J., Irudayaraj, J., & DebRoy, C. (2007). Direct detection of E. coli O157: H7 in selected food systems by a surface plasmon resonance biosensor. LWT-Food Science and Technology, 40(2), 187-192.
[21] Bergwerff, A. A., & Van Knapen, F. (2006). Surface plasmon resonance biosensors for detection of pathogenicmicroorganisms: Strategies to secure food and environmental safety. Journal of AOAC International, 89(3), 826-831.
[22] Xu, M., Wang, R., & Li, Y. (2016). An electrochemical biosensor for rapid detection of E. coli O157: H7 with highly efficient bi-functional glucose oxidase-polydopamine nanocomposites and Prussian blue modified screen-printed interdigitated electrodes. Analyst, 141(18), 5441-5449.
[23] Abdelbasset, W. K., Savina, S. V., Mavaluru, D., Shichiyakh, R. A., Bokov, D. O., & Mustafa, Y. F. (2023). Smartphone based aptasensors as intelligent biodevice for food contamination detection in food and soil samples: Recent advances. Talanta, 252, 123769.
[24] Kim, Y., Ma, L., Huang, K., & Nitin, N. (2023). Bio-based antimicrobial compositions and sensing technologies to improve food safety. Current Opinion in Biotechnology, 79, 102871.
[25] Achmad, H., Al-Hamdani, M. M., Ali, S. A. J., Kareem, A. K., Al-Gazally, M. E., Ramírez-Coronel, A. A., ... & Ali, S. H. J. (2023). Recent advances of amino acid-based biosensors for the efficient food and water contamination detection in food samples and environmental resources: A technical and analytical overview towards advanced nanomaterials and biological receptor. Microchemical Journal, 108552.
[26] Zhao, Z., Lu, M., Wang, N., Li, Y., Zhao, L., Zhang, Q., ... & Ma, L. (2023). Nanomaterials-assisted CRISPR/Cas detection for food safety: Advances, challenges and future prospects. TrAC Trends in Analytical Chemistry, 117269.

