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PROCESS OF DETECTING FOODBORNEPATHOGENS BY DIFFERENT METHODS

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ABSTRACT

The persistence of dairy-product-related food poisoning incidents in the United States highlights the need for quick ways of detecting pathogenic microbes in food processing settings. Salmonella and other pathogenic germs are common and may be found in a broad range of places, including meals, ingredients, and even industrial settings. Dairy products tainted with microorganisms pose a serious threat to public health since they raise the likelihood of infection and lower the standard of the food supply as a whole. The quick detection of important pathogens including Salmonella, Listeria monocytogenes, and Escherichia coli O157:H7 in contaminated foods and industrial settings has led to a focus on adapting or creating sensitive approaches. Even though the standard tests used to identify pathogenic bacteria are efficient, they may take several days to perform. Rapid, sensitive, automated processes are a major focus in food processing as a result of the pressing need to rapidly screen goods and surroundings in the industrial sector. There are a variety of detection and identification systems now in use within the food processing industry.

Keywords: - Food, Industry, Human, Foodborne,Bacteria.

I. INTRODUCTION

Preserving foods for use at a later date has been an integral element of human existence since the beginning of time. Some of the oldest and most reliable techniques for preserving food include salting, drying, fermenting, and heating. Since then, our understanding of science has increased, resulting in much lower food waste due to spoilage and contamination.

The microflora in processed and stored foods may be predicted based on their composition, storage conditions (such as temperature and environment), and the characteristics of the most common and resistant microbes. Technology (such as pasteurization and sterilisation), hygiene strategies (such as good hygiene and good manufacturing practises), and traceability (to prevent and reduce the distribution of unsafe and poor quality food) can all be used to avoid and delay spoilage and pathogenic microorganisms in food. Nonetheless, pathogenic microorganisms can

contaminate and spoil food at any stage of the food supply chain, including grocery stores, restaurants, and individual homes.

Businesses in the food industry might lose customers and face legal repercussions if their products cause illness. Consumers will abandon a product if its sensory characteristics have diminished, which may be related to microbial activity degrading the meal. Spoilage caused by microorganisms (such bacteria and molds) may be identified by the presence of slime or colonies, as well as by changes in texture caused by the breakdown of proteins, carbohydrates, and lipids.

However, pathogenic bacteria can spread, contaminate, and even produce toxins, jeopardizing food safety and putting consumers at risk. Diarrhea, abdominal discomfort, nausea, vomiting, and a high fever are all signs of gastroenteritis, which is caused by pathogen germs colonizing the human digestive system. International, national, and local surveys are required to calculate the global prevalence of foodborne illnesses. Although bacteria are the most common causative agents of gastroenteritis, parasites, viruses, yeasts, and molds have become increasingly important in the last decade.

Over the past two decades, foodborne illness has become increasingly common, making it a major global health concern. Because some cases are underreported, especially in underdeveloped nations, it is difficult to quantify the global incidence of foodborne diseases. However, there have been rising cases of food-borne illness in many regions of the world. There is a large range of microorganisms that may cause food-related illness. Pathogenic bacteria and viruses found in food are the leading cause of foodborne illness outbreaks worldwide. Norovirus, hepatitis-E virus, staphylococcus aureus, salmonella, campylobacter, and listeria monocytogenes are the most common pathogens associated with food-borne outbreaks.

II. DETECTIONOFFOODBORNEPATHOGENSUSINGPAPER-BASEDANALYTICALDEVICES

The necessity to promptly identify these organisms while keeping low cost is increasing because of the persistence of noteworthy foodborne pathogens across a wide range of food items and processing conditions. It may take several days for a verified result to be discovered using conventional culture procedures, which are widely regarded as the "gold standard" of detection and enumeration (Jokerst et al., 2012). Timeframes vary widely depending on variables like as pre-enrichment, enrichment, and selective plating. Since the product may already be in the hands of consumers by the time a conclusive result is obtained, the lengthy timeline poses a problem (Jokerst et al., 2012). Researchers have considered low-cost diagnostic (LCD) instruments as a means of speedy, sensitive, and selective detection to address this issue. Analytical devices

(PADs) made of paper are one such example. Wax printing is presently used because it is the fastest and simplest approach to make PADs (Jokerst et al., 2012). The cost of a single 8.5 × 11-inch sheet of Whatman® #1 filter paper is projected to be $0.001 per square centimeter, making wax printed paper a cost-effective option. Detection, enumeration, and identification procedures now used in industry are prohibitively costly compared to the cost of printing as many as 275 devices on a single sheet (about $0.002 per device; Jokerst et al., 2012).

Colorimetric readings are widely used in conjunction with paper-based analytical instruments to provide a graphical depiction of detection. To visually detect the presence of bacteria, an enzyme will react with a chromogenic substrate (Jokerst et al., 2012). Different chromogenic substrates with varying degrees of selectivity can be used for these kinds of tests. For the detection of Escherichia coli, for instance, a red-violet colorimetric product is created when chlorophenol red - galactopyranoside (CPRG) is degraded by -galactosidase. In the detection of L. monocytogenes, phosphatidylinositol-specific phospholipase C (PI-PLC) interacts with 5-bromo-4-chloro-3-indolyl-myo-inositol phosphate (X-InP) to produce a blue product, while in the detection of Salmonella, esterase interacts with 5-bromo-6-chloro-3-indolyl caprylate (magenta caprylate) to produce a purple product (Jokerst et al., 2012). Although PI-PLC is involved in the expression of virulence in pathogenic strains of Listeria, it retains a high degree of specificity with L. monocytogenes (Jokerst et al., 2012; Notermans, Dufrenne, Leimeister-Wachter, Domann, and Chakraborty (1991); Wei, Schreiber, and Baer (2005)). Non-specificity of the test has been reported due to the fact that E. coli O157:H7 is not the only E. coli serovar that produces - galactosidase (Jokerst et al., 2012). The enzyme -galactosidase is present in many different E. coli serovars and plays a crucial role in the hydrolysis of -galactosides into simple sugars. Thus, more research is needed to detect and identify additional serovars (Jokerst et al., 2012).

III. DETECTIONOFFOODBORNEPATHOGENSUSINGBACTERIOPHAGES

At first look, less-explicit approaches of microbial detection, such as the use of non-specific enzymes in the detection of foodborne pathogens, seem preferable. These techniques, such as nucleic acid amplification, can be extremely specific in their detection of a target organism while still being as sensitive as traditional techniques. However, there are a few issues with these approaches that make them less desirable than PAD colorimetric detection: they take longer, are more tedious, and can cost more. However, in manufacturing processes where identification and detection of an organism of interest is crucial, such as in meat, poultry, and dairy processing plants, the lack of specificity using ALP and oxidoreductases can be detrimental. However, researchers have turned to bacteriophages as a sensitive and specific low-cost alternative to

microbial detection (Hice et al., 2018) to address this lack of specificity during detection. Infected bacteria may be replicated and killed by bacteriophages, which were discovered in 1915 (Entis et al., 2001). Bacteriophages, which are made up of protein and either DNA or RNA, need a host bacterium in order to reproduce since they hijack the bacterial cell's replication machinery. Bacteriophage replication requires the host cell to maintain site-specific receptors for a particular bacteriophage; such receptors can include lipopolysaccharides, proteins, pili, flagella, and teichoic acids, among others (Entis et al., 2001), making it a challenging target for detection. Bacteriophages are essential parts of inexpensive detection technologies because to the site-specific receptors they use to retain host specificity to genus (Entis et al., 2001).

E. coli and Salmonella, among others, may be detected and captured using a combination of tetrazolium salts and bacteriophages (Hice et al., 2018). These techniques use the bacteriophage's reactivity and durability to provide a low-cost, portable test for microbial identification by adsorbing it onto paper (Hice et al., 2018). The capacity of bacteriophages to catch bacteria has been studied before (Anany, Chen, Pelton, & Griffiths, 2011). Specifically, bacteriophages have been used to manage the spread of E. coli O157:H7 and Listeria monocytogenes in food processing facilities (Anany et al., 2011). This was accomplished by binding bacteriophages specific to these organisms to positively charged cellulose membranes (the negatively charged head of the bacteriophage interacts with the positive cellulose membrane), and the results were observed in bacteriophage-treated ready-to-eat (RTE) meats that showed control and reduction in bacterial growth (Anany et al., 2011). Salmonella and E. coli O157:H7 are only two examples of foodborne pathogens that have been the focus of extensive research into bacteriophage-based detection methods (Favrin, Jassim, & Griffiths, 2003). Immunomagnetic separation (IMS) allows for precise binding to target cells using antibody-coated magnetic beads (Entis et al., 2001; Favrin, Jassim, & Griffiths, 2000), and has been used to capture and concentrate target organisms in certain cases. The cells are concentrated and purified from the food matrix when the beads are grabbed by a magnet (often positioned on the side of the tube) (Entis et al., 2001). By using IMS instead of time-consuming enrichment steps, assay times can be cut by up to 24 hours, and the purified mixture can be easily coupled with a wide range of detection methods like ELISAs and nucleic-based amplification techniques (Entis et al., 2001; Favrin et al., 2000; Favrin et al., 2003).

IV. MULTIPLEXDETECTIONOFFOODBORNEPATHOGENSBYREAL-TIMEPCR

Though effective in identification, the most frequently used assays for detecting foodborne pathogens can take up to seven days to process, which can impede high-capacity processes and lead to higher costs and more waste (Omiccioli et al., 2009). So scientists are trying to figure out

how to detect substances quickly using analytical methods (Omiccioli et al., 2009). Polymerase chain reaction (PCR) is one such technique widely used in business. Established in 1983, PCR is not a "new" detection technology; rather, it serves as the foundation for other bacterial detection methods in both industry and research (Entis et al., 2001; Lazcka, Del Campo, &Muoz, 2006). The polymerase chain reaction (PCR) is a fast DNA-based detection technology that can isolate, amplify, and quantify short DNA sequences in as little as five to twenty-four hours (Lazcka et al., 2006). Denaturing the target dsDNA at high temperatures (94-98 °C) separates the two strands, allowing PCR to proceed (Entis et al., 2001; Lazcka et al., 2006). DNA polymerase, an enzyme, adds nucleotide bases (dNTPs) onto the ends of each primer after specific primers anneal to complementary target DNA strands at lower temperatures (48-72 °C) and serve as templates for extension (68-72 °C) (Entis et al., 2001; Lazcka et al., 2006). During this process, two new strands of double-stranded DNA (dsDNA) are synthesized and used as templates for subsequent PCR cycles, resulting in exponential DNA amplification that can be observed (Entis et al., 2001; Lazcka et al., 2006).

Food-borne infections may be identified using a number of different techniques, including PCR-based approaches that are both sensitive and quick (Bhagwat, 2003; Omiccioli et al., 2009). Compared to traditional detection methods, the simultaneous detection of many prevalent foodborne pathogens may be more cost-effective since fewer analyses are required (Kawasaki et al., 2005). Multiplex polymerase chain reaction (mPCR) makes it simple to detect several targets in a single amplification process, and it may be used in tandem with Real-Time or quantitative polymerase chain reaction (qPCR) methods (Omiccioli et al., 2009). Analysis times may be drastically cut with these methods since there is no longer any need for post-PCR sample processing or concern for cross-contamination (Omiccioli et al., 2009). When the fluorescent dye binds to the target amplicon, as reported by Bhagwat (2003) and SYBR Green I (Lazcka et al., 2006), fluorescence emission occurs. Observation of DNA amplification in "real time" is made possible by measuring the fluorescent signal by optical detection of amplified targets, which in turn indicates the presence of double-stranded DNA or other dual-labeled probes (Lazcka et al., 2006; Omiccioli et al., 2009).

Studies have shown the need of an enrichment phase to boost sensitivity in order to properly identify the presence of foodborne pathogens. Non-specific products were detected without enrichment (Singh et al., 2011) and bacterial presence in contaminated milk samples was inconsistent (Omiccioli et al., 2009). After an enrichment step was implemented, the LOD for the various pathogens tested (Salmonella spp., Listeria monocytogenes, and Escherichia coli O157) was determined to be 1 CFU per 25 mL and 1 CFU per mL, respectively (Omiccioli et al., 2009; Singh et al., 2011). When compared to the seven or more days required by traditional culture techniques, the processing time was reduced to 48 hours (Omiccioli et al., 2009).

V. CONCLUSION

Methods for determining the metabolic status of Salmonella Typhimurium and Escherichia coli in environmental samples are described using paper-based enzymatic colorimetric assays in Chapter 2 of this Study. The metabolic status of a bacterial cell determines the relative abundance of non-specific enzymes such oxidoreductases and alkaline phosphatases. These enzymes convert purple formazans from tetrazolium salts and yellow para-nitrophenol from para-nitrophenyl-phosphate. Within 60 minutes, a color change was evident on paper using the newly developed INT-PMS (metabolically active) and INT-PNPP (metabolically inactive) assays. Bacteriophages P22 and T4 are selective for either Salmonella or E. coli, allowing for highly targeted detection. We have developed a method for the semi-quantitative assessment of the quantity of bacteria in each metabolic stage that is affordable, portable, and quick (30 minutes) by employing non-specific bacterial enzymes to identify bacterial pathogens. The assays allow for the quick evaluation of the metabolic condition of bacteria, which is crucial for limiting the unfavorable impacts of rapid bacterial growth on the environment and industry.

The detection of Salmonella Typhimurium in aqueous food samples using a combination of magnetic ionic liquids (MILs) and recombinase polymerase amplification (RPA) is described in Chapter 3 of this Study. Since their use in the extraction and recovery of live Salmonella had not been previously reported, Ni(II)- and Co(II)-based MILs were investigated. Cells were preconcentrated to around 12 times their original concentration in about 5 minutes when Ni(II) and Co(II)-MILs were used. After extraction, a 20-minute isothermal RPA test was performed, and gel electrophoresis or nucleic acid lateral flow immunoassay (NALFIA) was used to visualize the findings. During the amplification process, sodium acetate heat packs were used as a chemical heat source. Our MIL-RPA-NALFIA methodology offers a low-cost, transportable, and quick (30 min) method for detecting bacterial pathogens. Users benefit from the simplicity and clarity of the single-tube detection method since it is straightforward to use and understand. This work is currently geared toward helping the food processing industry by facilitating quick in-plant testing of foods and environments.

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