RAPID DETECTION AND CHARACTERIZATION OF FOODBORNE PATHOGENS BY MOLECULAR TECHNIQUES pdf

1: Methods for Rapid Detection of Foodborne Pathogens: An Overview

In recent times, several foodborne pathogens have become important and a threat to public health. Surveillance studies have provided data and a better understanding into the existence and spread of foodborne pathogens. The application of molecular techniques for detecting and typing of foodborne.

Conventional methods used traditionally for microbial analysis are regarded as gold standards except for time delay and labor involved. Many attempts have been made to improve laboratory efficiency by making the procedures for traditional agar based methods more convenient, user friendly and to reduce the cost of material and labor. Several modifications in sample preparation, plating techniques, counting and identification systems have made these conventional methods faster and easier Vunrcrzant and Pllustoesser, ; Doyle, Gravimetric diluters-automatically adds the correct amount of diluents to the test sample before homogenization. Massages samples in a sterile disposable bag eliminating need to sterilize and to use blender cups. This apparatus beats the outside of a sterile disposal bag at high frequency rpm producing a combination of shock waves and intense stirring which drives the microbes into suspension. There are several methods of adding sample homogenate to the agar plates. Spiral plater-this deposits a small volume on to the surface of the agar in a spiral fashion such that there is a dilution ratio of from the centre to edge of the plate. The colonies appearing along the spiral pathway can be counted either manually or electronically. As the volume dispensed at any point is known, this technique eliminates the need for serial dilution before plating and less time requited for colony counting. The agar slides containing selective or non selective media are pressed on to the surface to be examined and replaced within a sterile sleeve. Use of flurogenic and chromogenic substrate: In selective media detection, enumeration and identification. This eliminates the use of subculture media and further biochemical tests. These compounds yield bright color fluorescent products when reacting with specific bacterial enzymes or metabolites. Flurogenic enzyme substrates are derived from coumarin, such as 4- methylnubelliferone, while chromogenic enzymes compounds arc mainly phenol derivatives. Alternative to agar poured plates, which consist of rehydratable nutrients that are embedded into a film along with gelling agent, soluble in cold water. One milliliter of liquid sample is placed on the centre of film system and the rehydrated growth of microorganism. After incubation, the colonies can be counted directly from the film system as in conventional plates. These petrifilm products are available for yeast and mould counts, TVC, coliforms and E. Works by confining colony growths 10 a set of grid cells. These techniques has the advantage of removing inhibitors or unwanted nutrients, concentrating organisms, as well as three log unit range. The food samples obtained are homogenized and pre filtered in nitrocellulose membrane filters, which trap food particles larger than micrometers. This filtrate is then filtered through HGMF, which traps target microbes. The inoculated HGMF are placed in suitable agars and colonies are counted after incubation. This process is time consuming and several attempts have been made to automate this last step in enumeration of microbes to improve efficiency and labor cost. Image analysis systems have been shown to be useful and cost effective. An image of the plate is stored and can be viewed, printed or imported to other programs. The user can set variables such as top or bottom lighting and colony size limit to exclude spreaders or background particulars. A variety of morphological, physiological and biochemical tests are used for identification of microorganisms in conventional methods. Now several commercially available kits have been developed to simplify and automate the identification of individual organisms, the result of which is comparable to that of conventional identification systems. Biosensors are defined as indicators of biological compound that can be as simple as temperature sensitive paints or as complex as DNA-RNA probes. The science of biosensor is a multidisciplinary area. The potential application of biosensor technology to food testing offers several attractive features. Many of the system are portable and hence can be used for field testing or on the spot analysis and are rapid test which are capable of testing multiple analysis simultaneously. Biosensing methods for pathogen detection are centered on four basic physiological or genetic properties of microorganisms:

Interestingly, many of the modern-day biosensor-based methods are developed utilizing one of the above four principles or combinations of some sort. However, antibody-based methods are the most popular because of their versatility, convenience and relative ease in interpretation of the data. It is interesting to note that a majority of biosensors use antibody for capture and detection of the target analyte Ritcher, Recent advances in bio-analytical sensors have led to the utilization of the ability of certain enzymes to emit photons as a byproduct of their reaction. This phenomenon is known as bioluminescence and may be used to detect the presence and biological condition of the cells. Among the emerging technologies for rapid microbiological analysis, this technique giving results in a short time. Two distinct areas of Bioluminescence are of use in food industry: All living cells contain the molecule ATP. This molecule may be analyzed simply using an enzyme and coenzyme complex Luciferase- Luciferin found in the tail of fire fly Photinus pyralis. The total light output of the sample is directly proportional to the amount of ATP present and can be quantified by luminometers. At least cells are required to produce a signal. This system lacks specificity, but because of rapid response time for obtaining results, this system is very suitable for on-line monitoring of HACCP programs. This technique has a detection limit of 1 pg ATP which is equivalent to bacterial cells. ATP is present in both non-microbial and microbial cells. To determine microbial ATP selective extraction is used. First, non-microbial ATP is extracted with non-ionic detergents and then destroyed with high levels of potato ATPase for 5 minutes. The gene responsible for bacterial bioluminescence lux gene has been identified and cloned. The DNA carrying this gene can be introduced into host specific phages. These phages do not posses the intracellular biochemistry necessary to express this gene, hence they remain dark. However, on transfer of lux gene to the host bacterium during infection results in light emission that can be easily detected by luminometers. This technique can detect 1 x cells in 60 min. The specificity of this assay depends on phage specificity e. Bacteriophage p22 is specific for Salmonella typhimurium. Fiber optic biosensor is one of the first commercially available optical biosensors, marketed by Research International Monroe, WA for the detection of foodborne pathogens. The basic principle of the fiber optic sensor is that when light propagates through the core of the optical fiber i. The waveguides are generally made up of polystyrene fibers or glass slides. When fluorescent labeled analytes such as pathogens or toxins bound to the surface of the waveguide, are excited by the evanescent wave generated by a laser nm and emit fluorescent signal Bhunia, ; Taitt et al. SPR is a phenomenon that occurs during optical illumination of a metal surface and it can be used for biomolecular interaction analysis. Receptors or antibodies immobilized on the surface of a thin film of a precious metal gold deposited on the reflecting surface of an optically transparent waveguide are used to capture the target analyte. The sensing surface is located above or below a high index-resonant layer and a low index coupling layer. When a visible or near-infrared radiation IR is passed through the waveguide in such a way, it causes an internal total reflection on the surface of the waveguide. At a certain wavelength in the red or near-IR region, the light interacts with a plasma or cloud of electrons on the high-index metal surface and the resonance effect causes a strong absorbance. The exact wavelength of this absorption depends on the angle of incidence, the metal, the amount of capture molecules immobilized on the surface and the surrounding material. The presence of ligands or antigens interacting with the receptor or antibody causes a shift in the resonance to longer wavelengths and the amount of shift can be related to the concentration of the bound molecules. SPR-based sensors are governed by two basic principles: Wavelength interrogation uses a fixed angle of incidence but measures spectral changes, while in angle interrogation, a fixed wavelength is used but the angle of reflectance is monitored. Most of the commercial SPR systems are operated based on the angle interrogation mode. SPR-based sensors allow real-time or near real-time detection of binding events between two molecules. The detection system is label free, thus eliminating the need for additional reagents, assay steps and time. The sensor can be reused for the same analyte repeatedly. It is highly sensitive and it can detect molecules in the femtomolar range Bhunia, ; Rasooly and Herold, Impedance microbiology detects microbes either directly due to production of ions from metabolic end products or indirectly from liberation of CO2. Microbial metabolism usually results in an increase in both conductance and capacitance, causing a decrease

in impedance. A bridge circuit usually measures impedance. This method is well suited for detection of bacteria in clinical samples and to monitor quality and detect specific food pathogens.

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2: rapidmicrobiology Molecular Testing for Food Pathogens

Traditional methods of identification of food-borne pathogens, which cause disease in humans, are time-consuming and laborious, so there is a need for the development of innovative methods for the rapid identification of food-borne pathogens.

Apr 1, End Date: Mar 31, Objective: Apply rapid and high volume centrifugal flow concentration to the separation of bacteria from food matrices. Partition and concentrate bacteria using immunomagnetic separation with a new class of antibody-coated paramagnetic particles. Compare and contrast bacteria separation and concentration with flow-through filtration systems. Develop and validate procedures for the rapid and quantitative detection of multiple foodborne pathogens. Generate portable, label-free sensors e. Develop a flow-through immunoelectrochemical detection device for field portable detection of target pathogens. Direct typing colony isolates not required of enriched samples using a targeted-sequencing method. Generate genome sequence-based typing and identification schemes using next-generation sequencing technology e. The primary objective of the plan is to develop rapid screening and identification methods for top foodborne bacterial pathogens, including STECs, top Salmonella serotypes, and Listeria monocytogenes as well as those of intermittent concern. Novel or enhanced sample preparation techniques e. Also, enhanced detection systems will be needed in order to bypass growth enrichment and achieve the desired, quantifiable detection levels. Furthermore, numerous biomarkers and the potential for false positive results using cross-reacting biorecognition elements will require multiplex detection techniques e. Methods will initially be developed with culture media or buffer as the sample matrix, and then extended to application with food primarily ground meat in multiple sample formats: Initially, this will be accomplished by reliance on enumeration of known bacterial isolates, quantified in pure culture with total cell counting if a significant dead population is expected. For evaluation, artificially inoculated and unknown samples will be tested with new methods as assessed against selective enrichment followed by selective and differential plate agar analysis. Regulatory-based methods, such as biochemical testing, multiplex PCR, and serotyping, and possibly whole genome sequencing, may be invoked for additional comparison. Our sister agency, FSIS, will provide guidance as to the parameters and specifics regarding acceptable validation of desired rapid bacterial detection methods. Eventually, testing will move to the field- first off-line and near-line, then in-line for some analysis platforms e. It is expected that multitudes of tests will be conducted given that most samples will be negative.

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3: Project : USDA ARS

Rapid methods are important for the rapid detection of foodborne pathogens in food products to prevent outbreaks of foodborne diseases and the spread of foodborne pathogens. Rapid detection methods are generally more sensitive, specific, time-efficient, labor-saving, and reliable than conventional methods.

Download ePub Microbial infections including bacterial and viral infections are potentially life-threatening and require rapid identification and characterization of the microorganisms. These steps are important for: Traditional microbiological detection starts with an enrichment step usually using a nonselective enrichment that allows injured cells to repair and multiply. These enrichment cultures are then subcultured on selective and differential agars. Typical and suspected colonies are then stained by Gram staining and subsequent biochemical identification, and susceptibility testing are performed. These steps usually take days. Identification of virulence factors requires additional time and involves expensive phenotypic and genotypic tests. Traditional microbiological identification methods often do not provide prompt results for optimal early patient diagnosis and treatment management. In recent years, more and more studies have demonstrated the value of molecular biologic techniques to rapidly identify and genotype bacteria or virus in food, environment, and clinical samples. Molecular biology techniques are methods which generally involve the analysis and handling of nucleic acid and other molecules. Compared to traditional microbial identification methods, molecular biology methods can be used to detect and identify microorganisms simultaneously, which results in quicker diagnoses and treatments. The other advantages of molecular methods are that the protocols are of higher sensitivity and specificity. The aim of this hot topic is to provide an overview of current exciting molecular biology techniques that are employed to rapidly identify and characterize important pathogenic bacteria and viruses. The topic would update knowledge on prompt bacterial and viral identification and characterization methods, which can speed up the diagnosis and treatment of infectious diseases, improve epidemiologic screening, and reduce their morbidity and mortality. Staphylococcus aureus causes a wide range of suppurative infections, food poisoning and toxic shock syndrome. MRSA infection is more difficult to treat with commonly used antibiotics and thus more dangerous. Clin Infect Dis ; 52 It is important to precisely and timely detect and identify the organism for epidemical and clinical investigation. Bacteroides fragilis is an anaerobic opportunistic pathogen, which cause bacteremia, abscess formation, diarrhea, and inflammatory bowel disease [2 Boleij A, Hechenbleikner EM, Goodwin AC, et al. The Bacteroides fragilis toxin gene is prevalent in the colon mucosa of colorectal cancer patients. Clin Infect Dis ; 60 2: Next generation sequencing and analysis tools can be employed to detect quickly B. The isolation, detection and characterization methods of Enterotoxigenic B. Francisella tularensis is a small, nonmobile, Gram-negative bacterium. Humans and evolutionary and ecological forces shaped the phylogeography of recently emerged diseases. Nat Rev Microbiol ; 7 Clin Microbiol Rev ; 15 4: A review article written by Lai et al. Noroviruses are now recognized as the most common cause of nonbacterial acute gastroenteritis and foodborne viral illness worldwide. N Engl J Med ; Noroviruses are extremely infectious; a few viral particles can initiate an infection. A major impediment of the identification and diagnosis of norovirus infection is the lack of an effective cell culture system or small animal models. Molecular techniques have been developed to detect the viruses. The molecular identified and diagnostic methods of noroviruses are meticulously outlined by Drs. The value of molecular biology technique has been demonstrated in identifying and characterizing bacteria and viruses in food, environmental and clinical specimens, and in hastening diagnostic and epidemiological screening procedures, which circumvent the problems of traditional culture methods. Thus, these review articles will updated knowledge on microorganisms, and provide useful and practicable guides for rapid identification and characterization of the bacteria and viruses.

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Rapid and accurate detection of foodborne microorganisms from food, environment and clinical samples requires characterization to the species and/or subspecies level as well as determining their antibiotic susceptibility.

Project Methods Experiments on detection methods development will combine immunofluorescent nanoparticles and flow cytometry. Four genera will be included in the assay development: Assay will first be developed by testing in a model system bacterial broth media , then a liquid food system milk and bottled water. Studies on the prevalence, genotype, and antimicrobial resistance will be based on a carefully planned and executed sampling scheme. Following sample collection, bacteria will be isolated, characterized, and tested for susceptibilities against different panels of antimicrobials. Data will then be analyzed and compared with data reported from national surveillance systems. Examined the prevalence of antimicrobial resistance in lactic acid bacteria LAB from food. Surveyed the prevalence and antimicrobial susceptibility of Vibrio in retail and Gulf Louisiana oysters, Campylobacter and Salmonella in retail poultry, and Staphylococcus aureus, including methicillin-resistant S. Developed and applied a loop-mediated isothermal amplification LAMP technique to detect and quantify Vibrio parahaemolyticus, Vibrio vulnificus total and virulent-type , and Salmonella spp. Evaluated the use of propidium monoazide PMA for the detection of live Salmonella cells in produce. Developed a multiplex polymerase chain reaction PCR assay to simultaneously detect and characterize V. Examined the survival and internalization of Escherichia coli O H7 into growing spinach plants through soil inoculation in a greenhouse setting. Investigated the fitness cost of macrolide resistance and the potential of an efflux pump inhibitor to double as a membrane permeabilizer in potentiating macrolides activity in Campylobacter jejuni. Food safety researchers, educators, and consumers. Food safety regulatory agencies and public health officials. Nothing significant to report during this reporting period. Impacts Characterizing antibiotic resistance in lactic acid bacteria contributes to testing the overall hypothesis that commensals serve as reservoirs for antimicrobial resistance genes. Surveying retail food products for the prevalence and antimicrobial susceptibility of important foodborne pathogens provide important assessment on the contamination problems at retail, which represents the point of contact closest to consumers. The development of rapid, specific, sensitive, and quantitative LAMP assays will provide important tools to assess the microbial safety of foods, so that the industry and regulatory agencies can provide timely control in case of contamination, therefore minimizing the food safety risks. This addresses the problem of false-positive results associated with molecular detection assays when used to detect Salmonella in produce. Characterizing Staphylococcus aureus strains for the presence of enterotoxin and other exotoxin genes and antimicrobial susceptibility testing will address two aspects of food safety concerns of this pathogen, one causing foodborne intoxication and the other one infection. Characterizing the surface contamination and internalization of E. Understanding the fitness cost and mechanism of an efflux pump inhibitor could facilitate the control of macrolide development in C. Analyzing four biomarkers of V. Using a multiplex PCR to simultaneously detect and characterize V. Development of a toxR-based loop-mediated isothermal amplification assay for detecting Vibrio parahaemolyticus. Multiplex PCR assays for simultaneous detection and characterization of Vibrio vulnificus strains. Letters in Applied Microbiology. Quantitative detection of Vibrio vulnificus in raw oysters by real-time loop-mediated isothermal amplification. International Journal of Food Microbiology. Characterization of toxin genes and antimicrobial susceptibility of Staphylococcus aureus isolates from Louisiana retail meats. Foodborne Pathogens and Disease. Two real-time loop-mediated isothermal amplification LAMP assays were developed and evaluated to detect Vibrio parahaemolyticus and Vibrio vulnificus. Assay characteristics including sensitivity, specificity, quantitative capability, and applicability in raw oysters were evaluated. The population structure of V. Multiplex PCR assays were developed to simultaneously detect and characterize V. One hundred and fifty-three Staphylococcus aureus including 23 methicillin-resistant S. The effect of an efflux pump inhibitor doubles as a membrane permeabilizer in

potentiating macrolides activity in Campylobacter jejuni was examined. A survey examining the microbial safety of imported and domestic seafood products in Baton Rouge retail stores was conducted. Both indicator and major foodborne pathogens were studied. Five graduate students, two M. Not relevant to this project. The assays could be executed on two real-time platforms, one was in a real-time PCR machine and the other one a real-time turbidimeter. The assays hold great potential for the rapid detection and quantification of these vibrios in oysters. This addresses the problem of false-positive results associated with common molecular detection assays such as PCR when used to detect Salmonella in produce. Additionally, multiple biomarker characterization could be adopted to better characterize the population structure of V. The LAMP assay is rapid, sensitive, specific, and quantitative. Understanding the mechanism of efflux pump inhibitor could facilitate the use of this agent as a macrolide potentiating agent against Campylobacter jejuni, a major foodborne pathogen. Examining the microbial safety of imported and domestic seafood products in Baton Rouge will generate valuable scientific data to assess the current microbiological safety status of imported seafood products in comparison with domestically produced seafood products. It will also benefit the Louisiana seafood industry in an effort to promote seafood products from the Louisiana Gulf. Characterization of clinical and environmental types of Vibrio vulnificus isolates from Louisiana oysters. Effects of plant maturity and growth media bacterial inoculum level on the surface contamination and internalization of Escherichia coli O H7 in growing spinach leaves. Journal of Food Protection. Fitness cost of macrolide resistance in Campylobacter jejuni. International Journal of Antimicrobial Agents. Advanced technologies for pathogen and toxin detection in foods: Journal of the Association for Laboratory Automation. Prevalence and antimicrobial resistance of Salmonella serovars in conventional and organic chickens from Louisiana retail stores. Prevalence and antimicrobial resistance among Campylobacter spp. The loop-mediated isothermal amplification LAMP assay was further developed for real-time detection of Vibrio vulnificus and Vibrio parahaemolyticus. The assay characteristics including sensitivity, specificity, and applicability in raw oysters were evaluated. A survey examining the prevalence of methicillin-resistant Staphylococcus aureus MRSA in retail pork and beef meats in Baton Rouge, Louisiana was conducted. The MRSA strains were characterized by multiple phenotypic and genotypic methods. The survival and internalization of Escherichia coli O H7 into growing spinach plants through soil inoculation in a green house setting was examined. Two rounds of experiments were conducted. The fitness cost of macrolide resistance in Campylobacter jejuni was characterized using a parent strain and its isogenic mutants by assessing noncompetitive growth rate, pairwise competitive growth rate, and the ability to tolerate a chilling process commonly used in poultry processing plants. Five graduate students, three M. When coupled with a real-time turbidimeter, the assay holds potential for real-time detection and quantification of these vibrios in a field setting. The high prevalence of S. Further studies at the farm and retail levels involving larger sample sizes over time are needed to better assess the presence of MRSA in raw meats and the risk to meat handlers and consumers. Analyzing three biomarkers in a collection of V. There was also a large percentage Among 60 spinach plant samples examined for internal leaf contamination, only one plant yielded positive for E. Surface contamination occurred occasionally and clustered between three to five weeks after germination. No surface contamination occurred among leaves younger than three weeks of age. H7 strain survived the entire cultivation period but with gradually reduced levels. The experiments demonstrated that the internalization of E. H7 into a growing spinach plant under greenhouse conditions was a rare event, but contamination did occur, primarily when the plants reached 3 weeks of age. The study provided important data to further assess the association between spinach age and the potential contamination of E. Comparing the parent C. Additionally, the mutants demonstrated slower growth rates average doubling time of min versus min for the parent strain. However, the mutants were equally competent in their abilities to tolerate the chilling treatment. The findings indicated that acquiring macrolide resistance was associated with an obvious fitness cost in C. Conversely, the ability of macrolide-resistant C. A rapid, sensitive, and specific molecular-based detection assay, loop-mediated isothermal amplification LAMP , was developed to detect Vibrio vulnificus in oysters. A survey examining the prevalence and antimicrobial

susceptibility of Campylobacter and Salmonella in retail chickens in Baton Rouge, Louisiana was conducted over a one-year period. A strain collection database was established. The pulsed-field gel electrophoresis technique was used for molecular typing of Salmonella isolates from retail chickens. DNA fingerprinting profiles of these isolates were collected. Three graduate students, two M. LAMP can be used for rapid, sensitive, and specific identification of V. Because of its isothermal format and unique amplicon detection technique via unaided eye, the LAMP assay holds potential for future field applications. Among chicken samples collected for the survey, the prevalence rate for Campylobacter and Salmonella was No significant difference in prevalence of either Campylobacter or Salmonella was observed among chicken types, either conventional or organic. A total of Campylobacter were isolated, consisting of Campylobacter jejuni, 28 Campylobacter coli, and 33 other Campylobacter. Among four antimicrobial agents tested, the highest resistance rate was observed for tetracycline

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5: Rapid detection and characterization of foodborne pathogens by molecular techniques.

The surveillance of foodborne pathogens in food industries has shown the urgent need for rapid and dependable methods to detect and characterize the organisms in food and environments of clinical.

Not stated Bang et al. This method is able to monitor the PCR products formation continuously in the entire reaction by measuring the fluorescent signal produced by specific dual-labeled probes or intercalating dyes. The fluorescence intensity is proportional to the amount of PCR amplicons Omiccioli et al. This non-sequence-specific intercalating dye emits little fluorescence and the fluorescence signal is enhanced when bound to the minor groove of the DNA double helix Fukushima et al. The reporter dye and the quenching dye are close to each other and this prevent the emitted fluorescence of the reporter Levin, A reporter dye is attached to one end of the probe and a quencher dye is attached to the other end. Both dyes are in close proximity which maintained by the hybrid stem, hence, no fluorescence is produced Leone et al. Molecular beacon produces fluorescence signal upon hybridization of the probe to its complementary nucleotide sequence in the amplicon. During hybridization, the probe undergoes spontaneous conformational change that separates the two dyes and this allow fluorescence to occur Leone et al. The detection of Salmonella in fresh-cut fruits and vegetables by molecular beacon qPCR targeting the invasion associated gene iagA was first reported by Liming and Bhagwat Furthermore, multiplex qPCR assay is also developed for the detection and quantification of multiple foodborne pathogens. Additionally, a simultaneous detection of Vibrio parahaemolyticus, Vibrio cholerae, and Vibrio vulnificus by multiplex qPCR using primers that target vmrA, zot and vuuA genes respectively was developed by Kim et al. The detection limits of the assay ranged from 1. This is required in order to differentiate the target products from the primer dimer formation. The primer dimer products have lower Tm values as compared to the amplicons Levin, However, SYBR green dye will bind to other non-specific reaction products which include primer dimers Madani et al. As for TaqMan probes and molecular beacons, they are sequence specific probes and they only bind to their target sequence, hence, primer dimers will not be detected Levin, Nevertheless, the sensitivity of PCR-based method is mainly affected by primer specificity, primer sequence and annealing temperature, rather than the choice of detection probe Klerks et al. Conventional PCR and multiplex PCR that require agarose gel analysis for the detection of PCR products are laborious and time-consuming, thus, not suitable for high-throughput analysis and difficult to automate Patel et al. This allows low risk of cross-contamination, high-throughput analysis and automation Fricker et al. The advantages of qPCR have led to the development of various commercial qPCR kits for the detection of foodborne pathogens such as Salmonella, Listeria monocytogenes, Escherichia coli O H7 and Campylobacter Maurer, PCR amplification and detection kits for the detection of Salmonella agona in artificially contaminated milk powder and ricotta cheese. Besides, Margot et al. Detection Kit Applied Biosystems. A total of 49 Salmonella strains were included in this study and correctly identified by all the evaluated systems. Besides, Wan et al. There are many more commercial qPCR kits that are available for the detection of other foodborne bacterial pathogens such as Escherichia coli and Campylobacter. The post-NASBA product detection methods such as agarose gel electrophoresis or enzyme-linked gel assay is considered labor-intensive and not cost-effective. Real-time NASBA has been used for the detection of various foodborne pathogens such as Salmonella enterica, Vibrio cholerae, Staphylococcus aureus, Campylobacter jejuni, and Campylobacter coli Simpkins et al. NASBA offers high-throughput analysis and it has been commercialized as kits. H7 Maruyama et al. Since then, LAMP has been used for the detection of various foodborne pathogens due to its rapidity and sensitivity. This is because LAMP uses four primers targeting six specific regions and it provides rapid amplification, greater yield of amplification products and lower detection limits than PCR assays Hara-Kudo et al. For example, the Loompamp detection kit Eiken Chemical is commercially available for the detection of foodborne pathogens such as Salmonella enterica Ohtsuka et al. Besides, different types of LAMP assays have been developed for the detection of foodborne

pathogens. The availability of real-time monitoring of LAMP amplification products by the presence of turbidity or fluorescence eliminates the need for staining with ethidium bromide and gel electrophoresis. Therefore, this allows high-throughput analysis along with its high sensitivity and specificity Yang et al. Oligonucleotide DNA microarray The recent progress in multi-gene detection technology includes the microarray technology Call et al. Microarrays were originally used for the study of gene expression, but oligonucleotide DNA microarray has been widely used in the field of foodborne pathogen detection. Microarrays are made up of glass slides or chips coated with up to hundreds of specific oligonucleotide probes and these probes are chemically synthesized short sequences range from 25 to 80 bp Severgnini et al. Each oligonucleotide probe is able to target a specific part of a gene sequence. In this method, the sample nucleic acid fragments DNA, mRNA or cDNA are labeled with fluorescent dye, and then they are denaturated to generate single-stranded fragments. These fragments will hybridize to the array through binding to their corresponding oligonucleotide probes. The results are obtained through the visualization of the fluorescence signal produced from the probe-sample complex. The fluorescence intensity is proportional to the concentration of each labeled nucleic acid fragment Lauri and Mariani, The detection of a specific serotype can be crucial especially for Escherichia coli, as this pathogen has different serotypes with different level of pathogenicity that ranges from harmless strain Escherichia coli K to deadly strain Escherichia coli O H7 Lauri and Mariani, Moreover, Wang et al. Some examples of these pathogens are Staphylococcus aureus, Listeria monocytogenes, Vibrio parahaemolyticus, Vibrio cholerae, Campylobacter jejuni, Clostridium perfringens, Shigella spp. DNA microarrays are commercially available but most of them are designed for gene expression analysis studies Rasooly and Herold, The commercial in situ-synthesized arrays are high-density microarrays where short oligonucleotide probes range from 20 to 25 bp are synthesized directly on the surface of the microarray. In addition, multiple probes per target are included for higher sensitivity, specificity and accuracy. These high density microarrays require special manufacturing and they are relatively high in cost Rasooly and Herold, ; Severgnini et al. Most of the commercial microarrays are not desirable for specialized application such as food microbial analysis or diagnostic laboratory because low to medium density array will serve as the ideal microarray platform that can provide reliable results without involving the use of complicated equipments and data management Rasooly and Herold, ; Severgnini et al. In this case, custom microarrays are available from the Department of Bioresources at Seibersdorf and other organizations. Custom microarrays are sensitive, specific and less expensive than commercial microarrays Mothershed and Whitney, ; Severgnini et al. Nevertheless, low-density microarray is commercially available. In general, DNA oligonucleotide microarray allows simultaneous identification of multiple foodborne bacterial pathogens. Biosensor-based methods Biosensor is an analytical device that consists of two main elements: The bioreceptor responsible for recognizing the target analyte can either be a: The transducer that converts the biological interactions into a measurable electrical signal can be optical, electrochemical, mass-based, thermometric, micromechanical or magnetic Velusamy et al. Biosensors are easy to operate and they do not require sample pre-enrichment, unlike nucleic-acid based methods and immunological methods which require sample pre-enrichment for concentrating the pathogens before detection Singh et al. The recent biosensors that commonly used for the detection of foodborne pathogens are optical, electrochemical and mass-based biosensors Zhang, ; Zhao et al. Table 2 Examples of the application of biosensor-based methods for the detection of various foodborne pathogens present in food samples.

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Written by one of the most prolific and respected researchers in food safety, this book describes molecular techniques for the detection and discrimination of major infectious bacteria associated with foods.

Develop rapid and effective means to separate and concentrate targeted pathogens from food matrices that can be coupled to very rapid detection methods such as real-time PCR. Optimize reagents, apparatus and conditions to achieve maximum speed and recovery with minimum detection limits. Develop DNA extraction methods providing rapid, efficient, unbiased recovery of inhibitor-free DNA from a variety of pathogens. Examine environmental factors and microbiological culture conditions affecting genotypes or phenotypes that are important for virulence, isolation, or detection of foodborne pathogens. Detection of foodborne threat agents model system- pathogenic Yersinia spp. Isolation and detection of foodborne pathogens maintaining mobile genetic elements. Enrichment of pathogens while maintaining mobile genetic elements. Develop protein- and nucleic acid-based methods for the multiplexed detection and characterization of food-borne pathogens. Protein-based microarray and other multiplexed methods for the analysis of foodborne pathogenic bacteria. Oligonucleotide-based microarray for multiple pathogen detection and characterization. Multiplex real-time PCR for multiple pathogen identification and quantification. Develop typing methods for pathogens of concern to associated food regulatory agencies. Develop Restriction Fragment Sequence Polymorphism method for typing. This project plan has multiple goals that are distinct yet may be combined to generate improved, rapid techniques for the analysis of foodborne pathogenic bacteria e. Culture enrichment conditions e. Novel biorecognition elements initially, single chain variable fragment antibodies fractionated from naive phage display libraries will be custom generated to improve accuracy of biosensor-based detection or phenotyping platforms e. In addition, an abbreviated restriction fragment sequence polymorphism method will be developed and assessed as a novel genotyping method. Promising technologies will be directed towards usage by food producers and regulatory agencies for food safety monitoring and follow-up investigations. Progress was made on all four objectives and their subobjectives, all of which fall under National Program , Component I, [Microbial] Pathogens, Toxins and [non-biological-based] Chemical Contaminants: Progress on this project focuses on Problem Statement 1. Substantial progress was made on every subobjective outlined in this project plan. The goal of this project is to develop accurate methods for the detection and identification ID of pathogenic, foodborne bacteria. For Objective 1A, we have developed a fast method for separating and concentrating bacteria from foods using leukocyte reduction filters commonly used in hospitals for blood component separation. For Objective 1B, we have optimized the extraction of unique DNA factors using in-house developed reagents. For Objective 2A, a simple, economical, and highly reliable test using select organic dyes was developed to promote the rapid detection and isolation of Yersinia spp. H7, and Listeria monocytogenes in soft cheeses. When growing bacteria in a lab, it is impossible to use a rapid method e. Several hundred commercially available restriction enzymes were assessed with ca. Therefore, further pursuit of the RFSP approach is not recommended. Preliminary results indicate that this is a promising approach and that a few combinations of restriction enzyme pairs could provide discriminatory power comparable to or better than PFGE. Developed methods for separating and concentrating pathogenic bacteria from various food matrices. Foods and harmless bacteria in foods can interfere with accurate detection of harmful pathogens. ARS researchers at Wyndmoor, Pennsylvania have developed rapid filtration and centrifugation methods that not only separate, but to also concentrate target pathogens Escherichia, Salmonella, Listeria, and Campylobacter spp. Food regulators and producers will benefit immensely from applying these techniques since testing for harmful pathogens would be streamlined in terms of expense and time. Ensuring the ability to detect harmful Yersina pestis YP in food. ARS researchers at Wyndmoor, Pennsylvania have developed methods that were used to study the stability of the plasmid during growth of YP in raw ground meats pork and beef. Producers and regulators may use this information to reduce the incidence of YP in foods.

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Developed a typing assay for Shiga-toxin producing E. Methods are necessary for the rapid identification of detection and identification typing of harmful bacteria in foods. Antibody and DNA typing identification microarray platforms have been developed on both glass and relatively inexpensive polystyrene plastic substrates. Total assay times were typically under 3 h with real-time non-growth enriched samples with relatively low detection limits. The published finding may arm bacterial typing labs and regulatory testing agencies with additional means for ensuring biosafety as well as biosecurity of foods. There is a need to replace current methods for the identification of harmful bacteria in foods since such testing often takes long days to weeks. Faster identification reduces has benefits ranging from shorter food product holding times to accelerated epidemiological investigations. Food regulators and producers may apply this technique for the identification of harmful pathogens.

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