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Rapid detection techniques of microorganisms

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Abstract
The microorganisms present in foods may be of different types some produce beneficiary effects in foods while others may cause spoilage These Foods can also form reservoirs for diseases, and sources of contamination and thus detection and control of pathogens, toxicity produced by their toxins and spoilage organisms are necessarily to be studied in food microbiology Therefore, the ability to detect, identify, and compute unwanted microorganisms and other components in food are important to the food industry as well as to the consumer. Furthermore, these identification and detection techniques are crucial in food-related disease outbursts for epidemiologic inspection and analysis. Conventional methods make use of nonselective pre-enrichment media, selective enrichment media, and substantiation via morphological, biochemical and serological tests. Therefore, these methods are laborious, time consuming and are not always completely reliable In the need of overcoming of these obstructions a number of alternative, fast detection methods have been developed for identification and enumeration of the food pathogens. This is of high significance for the food industry since it requires faster methods for achieving of the ample result on possible presence of food borne pathogens in the production control and monitoring of hygienic practice in the facilities. These fast methods provide timely detection and enumeration of microorganisms and can be divided into modified and automated conventional methods, bioluminescent, immunological and molecular methods.

Keywords: Food borne pathogens, rapid detection, biosensors, assays

Introduction
Foodstuffs and microorganisms have extended and remarkable sorority which developed long before. Foods are not only nourishing to consumers, but are also excellent source of nutrients for microbial growth. The microorganisms present in foods may be of different types some produce beneficiary effects in foods while others may cause spoilage or may even preserve some foods by processes like fermentation process. Microorganisms can be used to convert raw foods into fermented products, including fermented milk products, cheese, fermented meat sausages, dill pickles, sauerkrauts, wine beers and other products. These Foods can also form reservoirs for diseases, and sources of contamination and thus detection and control of pathogens, toxicity produced by their toxins and spoilage organisms are necessarily to be studied in food microbiology. In the entire sequence of food handling from the manufacturer to the destined consumer, microorganisms can impinge on food quality and health. The contests in food safety have augmented due to the development of new products and production methods, globalization of the markets, increase in consumer knowledge, and to the high demand for different types and healthier foods. Consumers have better understanding of the contents of foods and demand for more consistent and more rapid processes to assure superior quality and attributes. They demand for foodstuff that contains less synthetic components, such as additives and preservatives, and also want foods safe in every context in regards to pathogenic microorganisms. Therefore, the ability to detect, identify, and compute unwanted microorganisms and other components in food are important to the food industry as well as to the consumer. Furthermore, these identification and detection techniques are crucial in food-related disease outbursts for epidemiologic inspection and analysis. Food borne illness is a prevalent crisis throughout the world. These illnesses caused by food borne microbial pathogens, include fungi, viruses, parasites and bacteria, that significantly affect people's health as well as being economically expensive. It has been anticipated that in the United States approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths will occur per annum (Mead *et al*. 1999) [35], which can cost approximately tens billions of US dollars (Buzby *et al*. 1996) [6]. In particular, food borne bacteria such as Escherichia coli O157:H7, Salmonella enteric, Staphylococcus aureus, Listeria monocy to genes, Campylobacter jejuni, Bacillus cereus, and other Shiga-toxin producing E. coli strains (non-O157 STEC), and Vibrio spp. are leading causes of foodborne diseases. In recent years, diseases caused by

Foodborne pathogens have become an important public health problem in the world, producing a significant rate of morbidity and mortality **(**Oliver *et al*.2005) [36] The high pervasiveness of foodborne diseases in many developing countries recommend major underlying food safety problems therefore, it is important to detect food borne pathogens in order to reduce food borne disease occurrence. Traditional methods for the finding of bacterial pathogens from foods depend on culturing the organisms on agar plates which is a protracted process, taking 2-3 days for results, and up to more than 1 week for validating the specific pathogenic microorganisms. It is perceptible that culture and colony counting methods are inadequate in order to prevent the spread of infectious diseases, ensure the food safety, and thereby to protect public health, there is an escalating demand for more rapid methods of food borne pathogen detection. Detection of food spoilage and pathogenic bacteria in food is a great task due to the verity that these microorganisms are present in low numbers in the food and are outnumbered by native bacteria. Conventional methods make use of nonselective pre-enrichment media, selective enrichment media, and substantiation via morphological, biochemical and serological tests. Therefore, these methods are laborious, time consuming and are not always completely reliable In the need of overcoming of these obstructions a number of alternative, fast detection methods have been developed for identification and enumeration of the food pathogens. This is of high significance for the food industry since it requires faster methods for achieving of the ample result on possible presence of food borne pathogens in the production control and monitoring of hygienic practice in the facilities. These fast methods provide timely detection and enumeration of microorganisms and can be divided into modified and automated conventional methods, bioluminescent, immunological and molecular methods (Fung, 2002 [16]; Scheu *et al*., 1998). Rapid methods can be assay or may be a simple modification of procedure that reduces the assay time. Rapid methods are also used in combination with traditional detection methods for enumeration of microorganisms which include isolates classification by microbiological methods, chemical methods, biochemical methods, immunological methods and serological methods or by combination of these methods.(Boeming and Tarr 1995; [5] yongshengh *et al* 1996;westerman *et al* 1997;griosman and ochman,2000; [18] biswas *et al* 2008) [4] The extent to which rapid methods and automation are expected and used for microbiological analysis is determined by the array and type of testing required volume throughput of sample to be tested availability of trained laboratory human resources and nature of manufacturing practices (vasvada 1993)

Methods of microbial detection in raw and processed foods

Conventional methods

These methods depend on media to enumerate and isolate microbial cells in food they are sensitive and can give both qualitative and quantitative information about the microorganisms in food (Doyle 2001) [12] Although these methods can be susceptible, reasonably priced and give both qualitative and quantitative information on the quantity and the disposition of the microorganisms tested, they are greatly constrained by assay time, with initial enrichment needed to detect pathogens, which typically occur in low numbers in food and water (LEONARD *et al*., 2003) [29] Food Products that are minimally processed have an naturally short shelf life, which prevents the use of many of these conventional methods. Therefore, broad research has been carried out over the years to diminish assay time through the use of substitutive methods for detecting foodborne microorganisms and reduce the amount of labor intensive methods by automated methods whenever possible (Jantzen *et al*. 2006 [24] a; Feng 2007; [14] Betts and Blackburn 2009; [2] Jasson *et al*. 2010) [25].

Rapid detection methods

Biosensors

Biosensor based equipments is the quickly emerging technology for pathogen detection in contrast to PCR, immunology, culture methods and gel electrophoresis.(Lazcka *et al*. (2007) [27] This technique is based on four assets of microorganisms which include, pattern of substrate utilization, phenotypic analysis of molecules by antibodies, nucleic acid analysis and the analysis of interaction of pathogens with cells. These devices for pathogen detection usually consist of three elements, including a biologically incarcerate molecule (e.g., probes or antibodies), a method of converting incarcerated molecule-target interactions into a signal, and a data output system (velusamy et.al 2010) [53]. For these purposes, pathogens are firstly secluded from the food matrix and then subjected to the analysis by biosensors. As the bio-receptor recognizes the target a biological response occurs and is converted into an electrical signal by the transducer. The electrical signal is passed to an amplifier where it is converted to an essential waveform, and passed onto a signal processor. (Velusamy *et al*., 2010).

ATP biosensor

ATP bio-luminescence was developed in the 1950s by NASA scientists. ATP bioluminescence is used to detect the amount of ATP produced by microorganisms, which is a circuitous measurement of the amount of food relics on a surface that has the possibility to support microbial growth ATP bioluminescenc was based on light produced by firefly. The firefly contains two chemical compounds, Luciferin and Luciferase, that react with the insect's ATP to produce bioluminescence light. The ATP reacts with Luciferin /Luciferase compounds in the sample swab to create bioluminescence light. The amount of bioluminescence light is calculated by the Luminometer and is expressed in Relative Light Units (RLU). RLU numbers are directly proportional to the amount of ATP, and therefore the amount of microbial biomass on the sampled surface. Bioluminescence produced by ATP may be used to compute the total microorganisms in a sample if the number of bacteria present is high (more than 10 4 CFU/g) then only it is applicable (Samkutty *et al*. 2001; [43] Jasson *et al*. 2010) [25] Results are provided in less than 5 min (Cunningham *et al*. 2011) [11].

Optical biosensors

Optical biosensors are leading alternatives to conventional time consuming methods due to their highly specified nature and cost friendliness and minute size. These sensors work on techniques including light absorbance, chemical luminescence, fluorescence, light polarization and rotation, and total internal reflectance (Terry and others 2005) [49] Optical biosensor make use of analyte binding induced changes in the optical properties of the sensor surface, which are then transformed to a detector. The optical biosensors operate by detecting an alteration in fluorescence, in absorbance or luminescence of the biosensor surface upon

analyte recognition. The main benefit of this technique is the timely, binding reaction and detection, consent to kinetic evaluation of affinity interactions and low instrument cost. Optical biosensors call for a appropriate spectrometer to trace the spectral chemical properties of the analyte. Mostly used optical biosensor is the fiber optic biosensor based on the principle of on propagation of light through the core of optical fiber which generates a transitory field outside the surface of the waveguide which is usually made up of polystyrene fibers or glass material when flourescent analytes i-e pathogens or toxins get bordered to the surface of waveguide they get excited by transitory waves generated by laser and radiate flourescent signal which travels back to be sensed by a fluorescence detector in minimum time fraction.(bhunia 2007,[3] taitt et.al 2006) [48]

Peizo Electric Biosensor

These are used for direct detection of microbial pathogens (Zourob *et al*.2010) [33].The basic principle is coating the surface of piezoelectric sensor with suitable and selective binding substance such as coating with antibodies is done for bacteria and then placing in the solution or sample containing bacteria, these bacteria bind to antibodies. The target nucleic acids of microganisms start to bind to their complementary oligonucleotides(antibodies) the mass of the piezoelectric biosensor increases with a proportional decrease in the resonance frequency of the quartz oscillation (Zourob *et al*., 2010) [33]. Piezoelectric quartz crystal microbalance (QCM) is the mostly used piezoelectric biosensor for pathogen detection. QCM biosensors have the advantage of immediate scrutinization, ease of use, biocompatible electrodes for ligand immobilization and label-free detection. Salmain et.al (2012) [42] used a QCM immuno-sensor for label-free detection of SEA, and they optimized a stratagem to immobilize antibody on the gold transducer and reported a detection limit of 0.194 mg/L in milk

Other rapid techniques

Direct epiflourescent filter technique

It is a tremendously rapid microbiological analytical technique, used for direct evaluation of microbial population size in diverse applications (Pettipher), The DEFT requires only 10 min of assay time for determination of microbial numbers in raw milk (Beck, et.al); elimination of overnight incubation is a major advantage. The cells are stained on the filter and examined under an epifluorescent microscope (Pettipher *et al*. 1992), In the direct epiflourescent technique, pathogens and other bacteria present in food substance are adsorbed on a filter membrane, which is painted with a fluorescent dye, that may be acridine orange, or any other suitable dye and is then examined by epifluorescence microscopic method. All the microorganisms are then rapidly detected and quantified. Convoluted method of fluorescent-antibody staining was adapted for specific enumeration of Salmonella to DEFT method. (Rodrigues 1990) and also for presence of Listeria monocytogen and other organisms in raw meats. (Sheridan 1991) [45]

Flow cytometry

Flow cytometry usually consists of a fluorescent microscope with cells flowing through the liquid. It comprises of four main elements: a light source fluid lines and controls (fl uidics), electronic network, and a computer (Longobardi Givan 2001) [31]. Flow cytometry quantitatively measures optical characteristics of cells when they are forced to pass individually through a beam of light. Fluorescent dyes can be used to test the viability and metabolic state of microorganisms (Veal *et al*. 2000) [52] Samples are injected into a fluid (dye), which passes through a sensing medium in a flow cell. The cells are carried by the laminar flow of water through a focus of light, each cell emits a pulse of fluorescence, and the scattered light is collected by lenses and directed onto selective detectors (photomultiplier tubes). These detectors transform the light pulses into an equivalent electrical signal which gives information of their size shape structure This technique is fast, automatic, and potentially very specific and gives result at rate of thousand cells per second.

Solid phase cytometry

It is a innovative technique for rapid detection of bacteria without need for growth phase (Heaese and Nelis 2002) [19] it combines aspects of both flow cytometry and epiflourescence microscopy. The sample to be tested for presence of microorganisms is filtered and the retained microoganisms are fluorescently labelled with argon laser dyes on the membrane filter and counted by a laser scanning device without human intervention. the entire membrane is scanned within minimum time and results are obtained within 3 minutes SPC in juxtaposition with fluorescent viability staining has also been reported as a device to detect viable but non-culturable Campylobacter jejuna (Cools *et al*. 2005) [10].

Electronic nose

The electronic-nose is used to detect volatile chemicals by an array of semi-conducting polymer sensors which facilitate the user to record aromas in a graphical and digital arrangement. It consists of sophisticated sensors, electronic pumps, flow controllers, data processing softwares. the microbial contamination is detected by the smell produced by bacteria for example some microorganisms produce gaseous compounds upon spoilage. the computer collects the odour signals, these signals are analyzed by software and then results are produced and analyzed Electronic Nose can be used to keep an eye on the microbial contamination of food by studying the pattern of volatile compounds produced by microorganisms during their metabolic processes.. The finger print changes can occur due to either the emergence of new chemical compounds (or metabolites) or may occur due to changes in the relative amount of the original volatile compounds. Compared to other time consuming conventional methods the electronic nose provide rapid and accurate ways of sensing the food contaminant bacteria with petite or no sample preparation.

Immunological methods

Immunological methods are based on antigen-antibody bindings. These assays depend mostly on the specific antibody- antigen binding. A large number of antibodies have been used in different assay types for detection of food-borne pathogens and toxins produced by them. The aptness of the antigen-antibody complex relies mostly on the antibodies' specificity. For the detection of food-borne pathogens by antibody-based methods, the influence of stress on antibody reactions should be scrupulously examined and understood first, as the physiological behavior in cells may often vary in response to a stress (Hahn et.al 2008) [20].

Lateral flow assays

Lateral flow devices provides a simple and rapid form of microbiological detection the result is indicated by color change, which is provided by enzymatic reaction. (Shim et.al 2007; [46] Park et.al 2008) [37] these usually include a dipstick made of a permeable membrane which contains colored latex beads or colloidal gold particles coated with detection antibodies aimed toward a specific microorganism. These particles are attached to the base of the dipstick, connected with the enrichment medium (Posthuma-Trumpie *et al*. 2009) [40] Depending on the presence of target microorganism in the sample, the colored reagent can become bound at the test line or zone (Gomez 2010) [17]. These are mostly designed to incorporate a visual response about 5-10 min after the sample is applied. This techniques provides accurate results with little or no instrumentation within minimum time (Feng P. 1997) [15].

ELISA (Enzyme-Linked Immunosorbent Assay)

ELISA is one of the extensively used assays for food-borne pathogen detection. In ELISA the antigens are detected by specific antibodies bound to an assay and a detection system indicates the presence of microrganisms and mass of antigen –antibody binding which is quiet similar to other assay detection techniques. Commercially most used method for ELISA assay is a "sandwich" assay. An antibody that is bound to a solid matrix is used to arrest the microorganisms and toxins (i-e antigens) from enrichment cultures and another antibody is bound to an enzyme and is used for detection. The enzyme is capable of creating a product detectable by a change in color, or in the case of enzyme-linked fluorescence assay (ELFA) in the form of fluorescence, which can be measured by using spectrophotometry of the antigen present in the sample which may be a microorganism or toxin produced by the pathogen (Cohen and Kerdahi 1996; Jasson *et al*. 2010) [25]. The results can be attained in 2–3 days instead of the 3–5 days or more given by conventional methods (Leon-Velarde *et al*. 2009) [30]. A complex sandwich immunoassay combined with IMS and fluorescent detection was reported by (Cho *et.al* 2014) for the highly sensitive detection of E. coli O157:H7 and S. Typhimurium within 2 h. ELISA for the detection of pathogens have limits from 10^3-10^5 cfu/ml^{-1} for cells of microorganisms and few nanogram (ng ml^{-1}) for toxins and proteins. Enrichment is required for direct detection of pathogens for atleast 16-24hrs.

Molecular detection methods

These are usually DNA based assays used for detection of pathogens and toxins present in foods.

PCR (Polymerase Chain Reaction)

PCR was discovered by Kary Mullis in 1985, and is considered as one of the innovative invention in recombinant DNA technology (Lorenz 2012) [32]. Molecular method, are becoming more popular due to their increased sensitivity. The most applicable method is Polymerase Chain Reaction- PCR. In the last century PCR has become prevalent method for food pathogen detection (Chen, 2003) [7]. PCR assays were used only in the research laboratories earlier but commercial PCR systems have been developed for food pathogen detection such are those for Listeria monocytogenes, E.coli O157: H7 and Salmonella sp. (Scheu *et al*., 1998). Being more specific in comparison to the conventional, method they are mostly preffered. The double-stranded DNA is denatured into single stranded, and specific primers or single-stranded oligonucleotides are annealled to these DNA strands, In the annealing step the synthetic oligonucleotide primers are added and binded to the single strands followed by extension of the primers complementary to the single stranded DNA, with a heatand temperature stable DNA polymerase in the presence of adenine, guanine, cytosine, and thymine. Repeating these steps, results in doubling of the initial number of target sequences with each cycle. This process is visualized as a band on an ethidium-bromide-stained electrophoresis gel. PCR is used even for detection of bacterial toxins by augementing specific genes that encode bacterial toxins. Toxins produced by organisms such as V. cholera, B. cereus, E. coli, and S. aureus have been detected by PCR technique. A large number of gene-specific hybridization probes have been designed to be used for t of toxin genes detection in foodborne pathogens (planche et.al 2008)[39]. L. monocytogenes detection was done by PCR in mayonnaise-based RTE salads including chicken, macaroni, potato, and seafood salads (Isonhood et.al2006) [21].

Multiplex PCR

It is the enhanced PCR method in which several targets can be detected all together in single reaction. Instead of using single set of primers, multiplex PCR uses multiple sets of primers each capable of detecting a gene, a gene variant, or a genomic marker of a specific organism, mPCR is beneficial as minimum time and exertion is required to produce the detection result of multiple target organisms. Milk and chicken meat matrices were used in multiplex PCR study to test the presence of *S. aureus E. coli* O157:H7 V parahaemolyticus, and Salmonella species. (Chiang et.al 2012) [8]. Without enrichment procedures, the detection limit of all pathogens rangedfrom10^2 to10^4 CFU/mL of food homogenates. Multiplex PCR assay was also used to detect E. coli O157:H7, B. cereus, V. parahaemolyticus, Salmonella spp., L. monocytogenes, and S. aureus in RTE food products including chicken, steamed pork hocks, oysters, fresh tomato juice, sushi, and lettuce (Lee et.al 2014) was also developed. All 6 pathogen were detected at 10^4 CFU/mL. or more in different food samples. Multiplex PCR can also be used to determine the structure of certain microbial communities and to evaluate community dynamics, during processes such as fermentation or in response to environmental discrepancy (Kong *et al* 2002) [26].

Real time PCR or Quantitative PCR

Quantitative OR real-time PCR is an advancement of general PCR, which monitors the formation and quantity of the augmented DNA products (Zhao et.al 2014) [55]. The assay offers simultaneous augmentation and detection of target DNA. Amplified DNA is then detected using a fluorescently labeled part of molecule which is detected by the thermocycler at every cycle (Dwivedi et.al 2011) [13]. Real-Time PCR technique is used for the unremitting collection and compilation of fluorescent signals from one or more PCRs. Generally two techniques are used to obtain a fluorescent signal from the amplification of product in PCR. In the first technique the innate properties of fluorescent dyes such as SYBR Green I, are used. As the dyes bind to ds (DNA) and it undergoes a change in shape, which increases their fluorescence (J. Logan *et al*., 2009) [23]. In the second method fluorescent resonance energy transfer (FRET) is used which depends on the presence of two molecules that interact with each other, where a one of the molecules has fluorescent properties. The fluorescent molecule is known as the donor, whilet he non-fluorescent molecule is known as the acceptor.

During fluorescent resonance energy transfer, the donor molecule is excited by an external source due to which it emits light at a, longer wave length that is used to excite the acceptor molecule. The signal comining from the acceptor molecule is detected by using the real-time instrument (Dorak 2006) [34]. A SYBR Green real time PCR assay was developed by (Fusco *et al*.) for identification and quantitative detection of S. aureus strains which harbor the enterotoxin gene cluster, regardless of their variants. When optimized quantitative PCR conditions were used, the assay was able to quantitatively detect at least about 10^3 and 10^4 CFU of the pathogen m^{-1} raw milk by the SYBR Green assay. One of the main advantages is that it collects data in the exponential growth phase and increase in dynamic range of detection, and no-post PCR processing (S. Padilla *et al*., 2009). It is currently used in the fields of viral quantitation, drug therapy efficacy, pathogen detection, and genotyping

Conclusion

Convectional food borne pathogen detection methods are quiet vulnerable, and are often too much time-consuming for use, and take days to a weeks to give results. Therefore, new methods that overcome the drawbacks of traditional methods are required. In recent times, several novel and rapid methods have been investigated and developed for the rapid detection of food-borne pathogens. Most of this novel method still require enhancement in features like sensitivity, selectivity, or accuracy to be used more efficiently and to provide rapid and accurate results in minimum time. The expansion in use of nucleic-acid-based assay methods and immunological methods has helped to get results in minimum time. Various rapid methods, such as nucleic-acid-based methods, immunological based methods, and biosensor-based methods and their combinations should be further exploited to increase their potentiality of performance for detecting different food borne pathogens and their toxicity. Most food-borne pathogens are mostly present in minimum numbers (<100 CFU/g) and thus there detection becomes a bit difficult. For this purpose such detection method should be brought in use which is quiet reliable, accurate, rapid, simple, sensitive, selective, and cost-effective would be perfect and pathogen detection would be easy in the food industry and related fields. Moreover, the trend of combining different methods together can create novel devices or methods to reinforce the benefits of rapid detection methods. If these methods are made applicable on broad base due to their great potential there is still a great chance for further developments in the future to provide better applicability and enhanced results

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