

Detection of bacterial pathogen using molecular techniques

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Aim: To review about the detection of bacterial pathogen using molecular techniques.

Objectives: To know more about the detection of bacterial pathogen using molecular techniques by understanding the research studies and collecting review articles.

Background: The application of molecular techniques for detecting bacterial pathogen provide reliable epidemiological data for tracing the source of human infections. A wide range of molecular techniques including pulsed field gel electrophoresis, multi locus sequence typing, deoxyribonucleic acid sequencing, repetitive extra genic palindromic have been detecting bacterial pathogen of great significance to humans

Reason: Molecular techniques are highly sophisticated and rapid method for detecting bacterial pathogen. Hence this review useful to know more about the detection of bacterial pathogen.

Introduction:

Accurate and definitive microorganism identification, including bacterial identification and pathogen detection, is essential for correct disease diagnosis, treatment of infection and trace-back of disease outbreaks associated with microbial infections. (1) Bacterial identification is used in a wide variety of applications including microbial forensics, criminal investigations, bio-terrorism threats and environmental studies. Molecular techniques are major tools for the analysis of microorganisms from food and other biological substances. The techniques provide ways to screen for a broad range of agents in a single test. It has truly come of age and its range of application is perceived to broaden in the near future. The food industries, water processors, and analytical laboratories have taken up the latter method; for rapid differentiation of species, strain identification and definition of strain relatedness from infected samples.

Molecular methods varies with respect to discriminatory power, reproducibility, ease of use, and ease of interpretation. (2-3)

Methods for detection of bacterial pathogen:

A rapid method is generally characterized as a test giving quicker results than the standard accepted method of isolation and biochemical and/or serological identification (4)). The demand for speedy results combined with major advances in a range of technologies has led to a vast array of rapid methods being developed and investigated over the past two decades. The use of such methods, either in a laboratory or for on-site testing of food production premises, depends not only on the time taken to get results but on a range of other factors such as robustness, sensitivity, specificity, accuracy, reliability, standardization, evaluation, throughput, ease of use, potential for automation, as well as cost, convenience, validation, and the throughput of the end user. The major advances in rapid methods have, in general, been in immunological and nucleic acid-based detection systems, although significant developments have been made and are being made in other areas including impedance and conductance, bacteriophages, biosensors, microscopy as well as in miniaturized, automated biochemical detection kits. (5) Many of the methods, as well as being used on their own for rapid detection, may also be used in combination, for example, immunomagnetic separation followed by bioluminescent detection. This has led to an almost exponential increase in the number of rapid methods being reported for detecting foodborne pathogens. (6)

Gene probe techniques:

The advent of gene probe techniques has allowed the development of powerful tests by which particular bacterial strains can be rapidly identified without the need for isolating pure cultures. The polymerase chain reaction (PCR) is a technique for in vitro amplification of specific segments of DNA by using a pair of primers. (7-8) A million-fold amplification of a particular region can often be realized, allowing, among a myriad of other uses, the sensitive detection of specific genes in samples. PCR can be used to amplify genes specific to taxonomic groups of bacteria and also to detect genes involved in the virulence of food-borne bacteria. The recently developed techniques for amplifying specific DNA sequences in vitro allow the detection of very small amounts of target DNA in various specimens. Theoretically these procedures can detect even one molecule of target DNA. By amplifying a sequence that is unique to the pathogenic micro-organism of interest, the in vitro amplification methods can be used to indirectly detect extremely low concentrations of microbes. (9)

Immunological assay

Immunological methods, in particular, have had a major impact on the development of rapid methods for food-borne pathogens and constitute the largest proportion of commercially available rapid tests for food pathogens. Immunoassays come in a variety of formats all of which involve the specific binding of antibody to an antigen of the food pathogen. (10) Latex agglutination is one of

the simplest examples, in which the antibody-coated coloured latex particles or colloidal gold particles specifically cross-link with antigens of the pathogen to give visible clumping, thereby enabling rapid identification of pure cultures isolated from foods. There are many commercial assays of this type for a range of food pathogens including *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* and *Shigella*, *Staphylococcus aureus*. (11)

The most common form of immunoassay used for food pathogen detection is the enzyme-linked immunosorbent assay (ELISA). Standard assays are based on an anti-body sandwich technique in which an antigen on the food pathogen in an enrichment culture bind to a specific antibody coated onto a solid support, typically a 96-well microtiter plate. Unwanted material is then removed by washing and detection of the pathogen is carried out by the addition of a second specific antibody coupled to an easily assayed enzyme (12) Enzymes commonly used are alkaline phosphatase, -galactosidase or horseradish peroxidase, which can be easily detected using colorimetric substrates. Alternative detection systems to enzymes are also in use with the sandwich assay format and these include fluorescence, or chemiluminescence, impedance, and polymerase chain reaction (PCR). (13-14)

PCR Methods

PCR is the most well-known and established nucleic-acid amplification technique for detecting pathogenic microorganisms. In this process a specific region of nucleic acid in the target organism is amplified up to a billionfold during a rapid three-step cycling process reaching easily detectable levels. Cell lysis is usually required to release the nucleic acid from the microorganism under detection and then, following denaturation of the target sequence, two short synthetic oligonucleotides or primers specifically anneal to complementary sequences on opposite strands of the DNA flanking the region of interest. The primers are then extended, using the DNA strand on which they have annealed to as a template. (15)The thermophilic enzyme Taq polymerase catalyzes the additional of complementary bases. Each cycle of denaturation, annealing, and extension results in a doubling in the amount of specific DNA of interest and, as all DNA produced at the end of a cycle then goes on to act as a template in the next cycle; there is an exponential increase in the amount of specific DNA generated. In conventional or block-based PCR, the accumulated DNA products are either visualized as a band on an ethidium bromide-stained agarose gel electrophoresis are identified on their base pair size (16) However, this method of detection does not confirm the PCR product is the specific gene fragment of interest only that it approximately has the expected number of base pairs. Further characterization such as through hybridization of PCR products to a specific probe or sequencing of PCR products would confirm they were the specific DNA region of interest. Some of the PCR detection methods based on the use of DNA probes can be performed in 96-well formats. For example, in a PCR ELISA system, the PCR-probe hybrid is captured in a 96-well plate and detected colorimetrically using a labeled anti-body. There are many PCR assays that have been described for food pathogens such as *Salmonella*, *E. coli* O157, *L. monocytogenes*, *Campylobacter*, and several others are commercially available. PCR offers a rapid, special, and sensitive method for the detection of food pathogens following enrichment, but strict measures, including the use of separate areas for performing different stages of the PCR process and the use of appropriate controls, must be in place to ensure that either samples or reagents do not become contaminated with amplified material. (17)

PCR assays can also be multiplexed to detect more than one specific target in one reaction, thus offering the potential of detecting several food pathogens in a single procedure. Primers for amplifying several different targets can be combined in one reaction and following PCR-specific amplicons detected on size difference by agarose gel electrophoresis or by using specific hybridization probes. Successful multiplex PCR assays depend on judicious primer design, to avoid primer dimer formation, and careful optimization of the PCR assay in order to prevent individual reactions having an adverse impact.(18-19)

Toxin detection:

There is a wide variety of microorganisms that are able to produce toxins. In this section, we discuss the detection of the toxins produced by *S. aureus*, *Vibrio cholerae*, *Clostridium botulinum*, *C. perfringens*, *Bacillus cereus*, and *E. coli*. A number of different types of methods have been developed for the detection of toxin genes and their toxic products. These methods include the detection of toxin genes by amplification methods and hybridization probing. (20)The toxins themselves have been detected using immunological assays, such as ELISA and agglutination tests, and by bioassays such as mouse neutralization testing and cytotoxicity assays in tissue culture. (21)

Conclusion:

The trend in pathogen detection is the continuing to move toward rapid methods for the detection and characterization of pathogens. This movement is facilitated by the desire to reduce the amount of time it takes to go from the collection of a sample to pathogen identification and characterization to food safety intervention. Ideally results will be ready in hours instead of days or weeks. A number of techniques have recently been developed with utility for use in identification and characterization, including immunological assays, real-time PCR assays, DNA microarrays, multilocus sequence typing (MLST), and multilocus variable number tandem repeat analysis (MLVA). (22-23)

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