Appraising Molecular Techniques for Rapid Identification and Quantification of Foodborne Pathogens

Review on emerging trends in rapid detection techniques for foodborne pathogen assessing three main topics real time nucleic acids based detection, bacteriophage –based biosensors, and advanced template preparation for PCR –based assays.

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Abstract: *Foodborne illnesses caused by bacterial pathogens poses a significant threat to public health worldwide. Traditional detection methods can take several days to produce results, making them inadequate for timely intervention in food safety issues. In recent years, significant advancements have made in the development of rapid detection techniques for foodborne pathogens. This review article provides an overview of the emerging trends in rapid detection technique for foodborne pathogens, including real time nucleic acid based detection, bacteriophage –based biosensors, and advanced template preparation for PCR based assays. The review highlights the advantages and limitations of these techniques and discusses recent advancements that have improved their sensitivity and specificity. The review concludes that these rapid detection techniques have the potential to revolutionize the way we detect and control foodborne illnesses, ultimately leading to a safer food supply and healthier communities.*

Keywords: Rapid detection technique, real time nucleic acid detection, bacteriophage, biosensors, PCR, assays, antibiotics, microorganisms, fluoropore labeled probes, piezoelectric

1. Introduction

Foodborne disease caused by bacterial pathogen gives a significant threat to public health worldwide. Rapid detection and identification of foodborne pathogen are significant in preventing and controlling foodborne disease. The detection method traditionally used can take several days or a week for producing the result, making them inadequate for the time for the safety of food issue. In fast few years, significant development in advancement of rapid detection techniques in food borne pathogen have been seen prominently. This technique depends on detection of specific nucleic acid, antibodies, or other biomolecules which are unique to another pathogenic microorganism. This review article aims to provide an overview on emerging trends in rapid detection technique for foodborne pathogen. Basically, the review will cover three main topics: real time nucleic acid - based detection, bacteriophage - based biosensors, and advance template preparation for PCR based assays. The first topic will cover the use of real time nucleic acid - based detection techniques, such as PCR and microfluidic devices, which have rapidly improved the speed and sensitivity of pathogen detection in the food sample. The second topic will cover the bacteriophage - based biosensors, which are highly sensitive and specific and will reduce the limitation rate found in traditional detection methods. Third topic will discuss about the advancement made in extraction and purification of DNA from food sample, which have improved the sensitivity and accuracy of PCR based detection methods.

Overall, this review will provide an insight about recent development in rapid detection technique for foodborne pathogen. This advancement has the potential to restructure the way we detect the way we control and detect foodborne illness, leading us to safer food supply and healthier community.

2. Comparison Between Review

Advance methods for rapid detection for food borne pathogens – There are various kinds of rapid detection, identification, and observation methods have been developed for food borne pathogens including nucleic acid - based methods, immunological methods, and biosensors - based methods, PCR based methods, bacteriophages - based methods which helps people detect various pathogen present in food materials used in da today life, for immediate detection. Some of these methods have high consumption of expanses, time and labor and covers a large space as the machines are large in size, they also needs a lot of preparation earlier the detection, whereas some of them are so fast and provide immediate results to the user in real time.

Various detection methods for detection of food borne microorganisms are listed below –
1) Real time nucleic acid based detection
2) Bacteriophage based biosensors
   - Advantages
   - Disadvantage
   - Future outlook
3) PCR based assays for detection of bacterial pathogens
   - Advantages
   - Disadvantage
   - Future outlook

1) Real Time Nucleic Acid based Detection methods for Pathogenetic Bacterial Detection in Food –

There are various nucleic acid - based detection instruments are developed and appeared in the market. These instruments and projects allow user the ability to amplify DNA or RNA as well as detect and confirm the target sequence identify in a closed tube format with the help of several flurophores, labeled probes, or both with or without them need to run gels. These flurophores labels help the pathogens to provide fluorescence which helps for the visualization and detection of these pathogens.

Real time nucleic acid based food pathogen detection is a leading - edge technology that has transformed the way to ensure the safety of our food supply. Fluropores labeled probes and with the addition of fluorescent dyes or marker, these probes light up like a beacons under special conditions providing more clear signals of the contamination, resulting a quicker response time to identify the hazardous pathogen in our food supply chain. The cutting edge approach allows for rapid and accurate identification of harmful pathogen in real –time. These probes are specially designed to target and bind with genetic material from targeted microorganism, allowing us to detect even a small amount of traces of contamination easy and quick. The procedure prevents overspill of various illness and guarantees consumer wellbeing in food product and manufacture.

However, the technology offers both advantages as well as disadvantages. One of the most significant advantages is fluropore labeled probes combines with DNA and RNA to target specific pathogens present in the sample. Additionally, real time instruments allows user to obtain results within few hours or a day. There are also some disadvantages to this approach, this requires highly trained technicians to handle the instruments and equipment properly. Besides this techniques rely on nucleic acid based methods rather than cell culture, they may give false positive response due to presence of dead cells still having genetic materials. This method will require a lot of labor and extensive sample preparation as well as it is time consuming. This sophisticated analytical method is expensive and depends on specialized instruments that require regular calibration maintained by trained professionals.

Flurophores labeled probes carries both advantages and disadvantage toward improving crop quality control process of food production facility, bringing challenges by experienced person giving consumers the confidence about what they are consuming.

2) Bacteriophage based biosensors for food –borne pathogen detection –

Biosensors have overcome the limitations that we can find in other techniques very prominently like time consumption and laborious, extensive sample preparation etc. It has improved the selectivity and integration on transduction of selective detection. It is actively used as recognition probes for pathogen detection. These biosensors are analytical devices which basically work as, it translates specific bio - recognition events into a measurable signals. It basically needs three components: *a sensor* function with the probe to give specificity of recognition, *a transduction* which gives signal to measure the signal to capture the analytes, *an amplifier* which amplifies the signal of capturing and convert them into data. There are several types of bacteriophage based biosensors, including optical, electrochemical and piezoelectric biosensors.

Optical biosensors make use of change in light absorption or fluorescence emission to detect the presence of bacteria. In these biosensors, bacteriophages are immobilized into surface and when the target bacteria bind to the bacteriophage, the change in optical activity of the surface is detected.

Electrochemical biosensors use changes in electric current or potential to detect the presence of bacteria. A case when a biosensor works, bacteriophage is affixed to the outer area of an electrode so that when the target bacteria bind themselves, a probable change in their electrical properties can outright be seen.

Piezoelectric biosensors carry out an immobilization of an explicit biological recognition element, for example, an antibody on the shell of a piezoelectric crystal. When target pathogen binds to the recognition element, it causes a change in the in the mass or density of the crystal, which in turn leads to a shift in its resonant frequency. The frequency shift can be measured and used to determine the presence and concentration of the target pathogen.

As it is bacteriophage based biosensors, it requires component such as bio probes for the search of genome of pathogen in the food sample.

Components can be used as bio probes are –
1) Nucleic acids – It is highly used as probes because of its ability to amplify a desired target DNA by its host using PCR.
2) Antibodies – Antibodies are highly used as bio probes because its ability of immobilizing quality of pathogens on the surface of wells in the biosensors.
3) Phages – phages (RBP) receptor binding protein, they generally focus on recognizing the carbohydrate or Protein sequences on the surface of host bacterium.

Biosensors offer numerous advantages over traditional analytical techniques. The number one being its highly valuable sensitive explicit detection of target molecules which traces the numerous analytes in each sample. The use of biosensors therefore, becomes very useful in wide arenas of applicability ranging from medical diagnostics to food safety testing.

The use of biosensors reduces the time and is cost - efficient as it requires minimal sample preparation making them convenient for field applications, where sample must be analyzed in limited time duration and limited resources. Biosensors are cheaper as compared to other analytical techniques, which further increases their availability.

Biosensors are also portable and can be used for real time monitoring. Making them particularly valuable for application such as continuous glucose monitoring in diabetes management. They require very less time to

generate result, which especially important in medical emergencies where time taking diagnosis can be critical. Overall, the flexibility, sensitivity and affordability of biosensors make them a very useful tool for wide ranges of purposes. Biosensors have minuses as well as their usage is quite powerful when it comes for detection and analyzation of innumerable biological elements.

One of the major drawbacks of biosensors is their complexity. Biosensors can be complicated and requires specialized knowledge to operate effectively. Proper training is essential to ensure that the result should be accurate another disadvantage of biosensors is their sensitivity. They can detect the smallest of elements but at the same time can produce false positive or negative results which can be problematic especially at the time when results become critically necessary.

Cost is also an important consideration while using biosensors. The production and maintain ace of biosensors can be expensive, particularly if specialized materials or equipment are needed. Biosensors also have limited target range. They are designed to detect specific substances, and their effect can be limited to those substances only.

Ultimately, regulatory approvals can be an obstacle for biosensors, particularly in food industry. Especially, when used for food industry it should meet the regulatory standards for accuracy, specificity and sensitivity. This can also be time taking and expensive process.

Overall, biosensors have their advantage as well as disadvantages. While they are powerful tools for detecting and analyzing biological substances careful considerations of their limitations is essential to ensure their effective and accurate use.

3) PCR based assays for detection of bacterial pathogens -
Polymerase chain reaction based assays have pathogen revolutionized the detection of bacterial pathogens these assays are highly sensitive and specific method for the identification of bacterial pathogens in clinical and environmental samples.

This method involves the use of specific primers that amplify a target DNA sequence of interest. The amplified product can be visualized using gel electrophoresis. The conventional PCR is a simple and inexpensive methods for the detection of bacterial pathogens. However, it has limitation in terms of sensitivity and specificity.

PCR based assays is the multiplex PCR. this method involves the simultaneous detection of multiple target sequences in a single sample. This method is useful in clinical settings where multiple pathogens may be present in a single sample. The detection of bacterial pathogen include nested PCR, reverse transcription PCR, each method have its advantage as well as limitation.

PCR assays have restructured the detection of bacterial pathogens their high sensitive and specificity. They are considered to be the best method for detecting bacterial infection in any sample one of the major advantages of PCR based assays in their sensitivity. This allows for the detection of very low levels of bacteria in a sample. The use of primers is designed specially to target the DNA of the pathogen to be detected. Thus, PCR based assays are able to detect bacterial pathogen even in complex situations where there are low levels of target pathogen. The PCR based assays have speed by which they provide accurate results. Different from the traditional methods which take several days or even weeks to generate results, PCR method produce results in just matter of hours.

This rapid turn about time can be difficult in situations where a quick diagnosis is needed to initiate appropriate treatment. The specificity of PCR based assays can accurately diagnose bacterial infections and help healthcare professional make informed decisions about appropriate treatment options. PCR based assays are very specific and distinguish between closely related bacterial species. This exclusivity can diminish the risk of misdiagnosis and ill - treatment.

Quantitative PCR based assays are useful in monitoring the progress of an infection or the effectiveness of the treatment. By measuring the amount of bacterial DNA present in the sample, these assays can provide information about the severity of the infection and whether or not the treatment is working. This information can be critical in guiding treatment decisions and improving patient outcomes.

Lastly, PCR based assays require minimal sample preparation, which makes them ideal for use in settings where resources are limited. The ability to perform PCR based assays with minimal sample preparation also reduces the risk of contamination and increases the reliability of results.

PCR based assays have remodeled the field of molecular biology as it enables the detection and amplification of small amounts of DNA or RNA. But these assays have their shortcomings. Major shortcomings being the occurrences of false positives. This can be because of contaminations during the assays process, or the presence of similar DNA sequences in the samples that are not the target of interest. False positives can be highly specific, leading to incorrect diagnoses, unnecessary treatments, and additional cost.

Another disadvantage of PCR based assays is their cost. PCR based assays can be expensive, especially if they are for large scale screening programs. It is recommended that the equipment used shall be ergonomically purchased to reduce the cost of maintenance.

The requirement for specialized equipment is another disadvantage of PCR based assays. PCR based assays require specialized equipment, including a thermal cycler, which is used to amplify the DNA or RNA. The thermal cycler is an expensive piece of equipment that requires regular maintainace and calibration. In addition, the PCR reaction must be carefully controlled to ensure accurate results.

PCR based assays limit information about the antibiotic resistance and virulence factors. PCR based assays are constructed to detect specific DNA or RNA sequences, and not provide critical information on other specifications of organisms where the susceptibility of such antibiotics or virulence's. In essence, additional tests are to be run to obtain a clear picture on the mentioned organism's characteristics.

3. Future Outlook

1) Multiplexed PCR allows for detection of multiple pathogens in a single time.
2) Next - generation sequencing is increasingly affordable, and these can provide information about the bacterial species, antibiotic resistance, virulence factors in a single test.
3) PCR based assays go well for point care testing improves the diagnostic testing.
4) PCR based assays can be combined with other technologies such as biosensors for faster and accurate detection.

4. Conclusion

In conclusion, the emerging trends in rapid detection techniques for foodborne pathogens, including real time nucleic acid based detection, bacteriophage –based biosensors, and advanced template preparation for PCR - based assays, have shown great promise in improving the speed, sensitivity, and specificity of pathogen detection in food. These techniques have the potential to significantly reduce the incidence of foodborne illness and improve public health and safety. As these technologies continue to develop and become more accessible, the food industry, public health organizations, and consumers can all benefit from their increased efficiency and accuracy.

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