CC-9: FOOD MICROBIOLOGY

Unit-9 :TRENDS IN FOOD MICROBIOLOGY

Dr. Lyned Dafny Lasrado

Asst. Professor

Department of Post Graduate Studies in Biochemistry

St Aloysius College, Mangalore-575002

Introduction

Food microbiology explores the fundamental factors affecting the presence, activity, and control of microorganisms in food. The subject also deals with the crucial concepts required to meet the minimum standards for food safety. Food microbiology includes wide range of concepts, which include, food production and processing using microbes (e.g. cheese, beer yogurt, wine etc), study of microbial induced food spoilage and detection and prevention of food borne diseases. In the recent years a drastic shift has taken place between the use of simple microbial plate count to the use of molecular biology techniques to assess and evaluate the quality of food. This shift is essential to live up to the changes that occurred in food industry, evolution of pathogens and the change in people'sbehavior's and susceptibilities.

This module is divided into the following sections and sub-sections

(1) Basicfood microbiology

1.1 Brief historical perspective

1.2 Factors affecting the growth of microorganisms in food

(2) Microbiological criteria

2.1 Indictors of microbiological quality

2.2 Food Safety and Standards Authority of India (FSSAI)

(3) **Culture methods used in food microbiology**

3.1 Quantitative methods

3.2 Qualitative methods

(3) Rapid and advanced techniques in food microbiology

4.1 Types of rapid techniques

4.2 Advantages and limitations of rapid and advanced techniques

4.3 Recent developments in detection techniques

(1) BASIC FOOD MICROBIOLOGY

1.1 Brief historical perspective

It is extremely difficult to pin down the exact beginning of human awareness of thepresence and role of microorganisms in food, however,the available evidence suggests that that this knowledgepreceded the establishment microbiology as a science. Following the discovery of the ubiquitous existence of microorganisms by Anton von Leeuwenhoek (around 1670s), some scientists startedexploring the possible association of microorganisms with food spoilage, food fermentation, and foodborne diseases. Loius Pasteur is credited with the major development of ideas on the possible rolesof microorganisms in foods.This paved the way for the establishment of early food microbiology in the 20thcentury. Some of the major developments in the field of food microbiology are briefly listed in Table1.

1.2. Parameters affecting the growth of microorganisms in food

There aretwo primary factors that affectthe growth of microorganisms in foodproducts: Intrinsic and extrinsic parameters.

Intrinsic parameters:areproperties that exist as part of the food product itself.

Examples of intrinsic parameters are:

1. pH
2. Moisture content
3. Oxidation-reduction potential
4. Nutrient content
5. Antimicrobial constituents
6. Biological structures

Extrinsicparameters: are propertiesof the environment (processing andstorage) that exist outside of the food product, whichaffectboth the foods and their microorganisms.

Examples of extrinsic parameters are

1. Storage temperature
2. Relative humidity
3. Presence/concentration of gases

4. Presence/activities of other microorganisms

(2) MICROBIOLOGICAL CRITERIA

Microbiological criteria are used to differentiate between an acceptable and unacceptable product/ food processing and handling practices. Microbiological criteria are used to assess the following; a) adherence to Good Manufacturing Practices (GMPs), b) the safety of food, (c) the maintenance of quality/ shelf life of certain perishable foods, and d) the suitability of food/ingredient for consumption.

The main objective of Microbiological criteria is to ensure the safety and quality of food. Microbiological criteria are an underlying component of any critical control point that address a microbiological hazard in Hazard Analysis and Critical Control point (HACCP) systems

2.1 Indicators of microbiological quality

Indicator organism can be used to determine either the microbiological quality or safety

Indicator microorganism - Indicators of quality

The indicator organisms of food quality should fulfill the following criteria

1. Should be present and detectable in the food where quality is being assessed
2. The growth / numbers should share negative correlation with product quality
3. Detected and enumeration should be easy and within a short time
4. Clearly distinguishable from other organisms
5. Their growth should not be inhibited by other background microflora

Indicator metabolites: In certain cases, a correlation is established between the presence of certain metabolic products and product quality loss. In these, the microbiological quality is determined by assaying the metabolic products

The indicator organisms/ metabolic products correlated with product quality are summarized in Table 2.

Indicator microorganism - Indicators of safety

Safety is determined by the presence or absence of pathogenic microorganisms and their toxins. Some of the most prominent food-borne pathogens include, *Salmonella* (multidrug resistant

strain), *Campylobacter jejuni, E. coli* O157:H7, *Listeria monocytogenes*, *S. aureus* MRSA, *Vibrio vulnificus*, *Yersinia enterocolitica*, *Arcobacter* spp. and *Mycobacteriumparatuberculosis.*

2.2 The Food Safety and Standards Authority of India (FSSAI): was established under Food Safety and Standards Act, 2006 which consolidates various acts & orders that have hitherto handled food related issues in various Ministries and Departments.

- FSSAI has been created for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption.
- The FSSAI is responsible for protecting and monitoring public health through the regulation and supervision of food safety
- The FSSAI is led by a non-executive Chairperson, appointed by the Central Government from amongst the persons of eminence in the field of food science or from amongst the persons from the administration who have been associated with the subject and is either holding or has held the position of not below the rank of Secretary to the Government of India

(3) CONVENTIONAL METHODS FOR DETECTION OF MICROORGANISMS IN FOOD

The conventional methods employed for the microbiological evaluation of food and food ingredients can be broadly categorized as quantitative and qualitative methods

3.1 Quantitative methods:are devised to enumerate indirectly or directly the microbial load in the test material.

Conventional culture-based methods rely on specific culture medium for the isolation and enumeration of bacterial cells, in five steps which include, pre-enrichment, selective enrichment, selective plating for colony identification, biochemical presumptive tests and serological confirmation.

3.1.1Plate count method:

The standard plate count or aerobic plate count (APC) method is commonly used to enumerate the total number of microorganisms in food products by determining the colony forming units (CFU). Most commonly, aliquots from selected dilutions are either pour plated or surface plated

on either nonselective/selective agar medium or nonselective/selective differential media. By modifying the culture medium and conditions of incubation, this method can be used to preferentially screen for group of microorganisms such as thermophiles, psychrophiles, mesophiles, proteolytic, resistant, lipolytic microorganisms etc.

Drawbacks of plate count method include (i) this method measures only live cells and therefore would not be of value to determine the quality of raw material used in heat processed food (ii) certain bacteria though present in low counts may be able to induce preponderant quality loss due to enhanced biochemical activity (iii) this method is of little value in evaluating the organoleptic quality of food.

3.1.2 Direct Microscopic counts (DMC):

This method is used to give an estimate of microbial cells in samples either counting stained cells under a bright field or live cells under a phase contrast microscope. The counts are expressed as microscopic counts per milliliter or gram food sample.

Drawbacks of DMC method are (i) the sample should contain relatively large number (> 10^5 CFU/ml) of microorganisms for effective use of this method, (ii)not recommended to differentiate between live and dead cells (requires the use of fluorescent dyes such as acridine orange) (iii) cannot effectively enumerate microorganisms in foods that have particles.

The use of DMC is restricted to a few products, such as raw non-grade A milk, dried milk, dried eggs and liquid and frozen eggs.

3.1.3 Most probable number (MPN):

Aliquots from serially diluted samples are inoculated in broth (tubes) containing selective agents (one or more), and incubated at recommended temperature and time. The broth tubes in each dilution are then scored for the presence and absence of growth. The number of tubes displaying growth in each of the three successive dilutions are correlated with the number of viable cells of the specific microbial groups using the available statistically calculated table.This method is known to give wide variations and is often used for the enumeration of coliforms in food and water by using brilliant green lactose bile broth.

*3.1.4 Dye reduction test**:***

This test relies on the principle that some dyes (eg. Methylene blue) are coloured in oxidized states but become colourless in reduced state under the influence of microbial growth and

metabolism. The assumption is that, the rate of reduction of the dye (specific concentration) added to food is directly proportional to the initial microbial load of the sample.

3.2 Qualitativemethods: are devised to determine whether a representative amount of food sample or a particular batch contains a particular bacterial species (generally pathogenic) among the total microbial population or not.

3.2.1 Isolation of pathogens:

This method aims at determining whether a particular sample does or does not contain spores or viable cells of specific pathogens. Some of the pathogens tested include, *Salmonella* spp., *Shigella* spp. and *V.cholerae*. This is normally considered as only a presumptive test which has to be followed by confirmatory test using advanced automated techniques.

3.2.2 Test for toxins:

Some pathogenic bacteria (eg.*S.aureus*) produce toxins in the food they grow causing food poisoning upon consumption. Microslide precipitation method is performed in which the extracted and concentrated toxin from the food sample is assayed against specificantibody.

(4) RAPID AND ADVANCED TECHNIQUES IN FOOD MICROBIOLOGY

Owing to the fact that conventional methods are labor intensive and lengthy, different rapid automated methods have been developed in the recent years to detect microbial load, food borne pathogens and their toxins.

In food microbiology, rapid methods include the newly developed techniques for detection of microorganisms or any modifications introduced into the conventionalmethod that ultimately reduces the analysis time. The advent of biotechnology has enormously altered food testing methods and numerous assays that are specific, faster and more sensitive than theconventional methods have been developed.

4.1Types of rapid techniques:

The common types of rapid advanced methods are (i) modification of conventional microbiological methods, (ii) nucleic acid-based detection, (iii) antibody-based detection, and (iv)biosensor-based Methods

4.1.1 Modification of conventional microbiological methods

This category comprises of any alteration/ modification of conventional assays that result in saving labor, time and materials. For example

c) Petrifilm test:

In this test disposable cardboard containing dehydrated media is usedfor the enumeration of total bacteria, specific bacterial species, or mold and yeasts. This test eliminates the requirement for preparing media and agarplates, and economizes in storage and incubation space.

b) Chromogenic and Fluorogenic substrates:

The introduction of chromogenic and fluorogenic substrates in special microbiological media provides a quick measure of specific enzyme activities which are characteristic traits of certain bacteria or bacterial groups. Example; Chromogenic substrate like o-nitrophenyl-β-D-galactoside (ONPG) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal)are incorporated in the culture media to measure the activity of β-galactosidase (GAL).

c) ATP Bioluminescence:

This technique measures (using luminometer) the light emitted by an enzymatic reaction between luciferin and luciferase that requires the presence of ATP. The amount of light emitted is proportional to the concentration of ATP, and therefore the number of microorganisms in the original sample.

4.1.2 Nucleic acid-based methods

Nucleic-acid-based food pathogen detection assays are highly specific, as they detect specific nucleic acid sequences in the test organism by hybridizing them to a synthetic oligonucleotide complementary to the specific nucleic acid sequence. Numerous nucleic-acid-based assays, including hybridization, amplification, microarrays, and biochips, have been designed for use as rapid methods to detect foodborne pathogens.

a)Polymerase Chain Reaction (PCR) based

Simple PCR Method:

In this method, double-stranded DNA is denatured into single strands, and specific primers are annealed to these DNA strands, followed by extension of the primers complementary to the single stranded DNA, with by *Taq*and other thermoresistant DNA polymerases (Fig 1). The

quantity of the products of amplification can be visualized as a band on an ethidium-bromide-stained electrophoresis gel. SimplePCR methodsfor toxin detection have been developed for a range ofbacterial species, such as *B. cereus, V. cholera*, *S. aureus*and*E. coli.*

Multiplex PCR: The method of rapid detection of multiple microorganisms in a single reaction by simultaneous amplification of more than one locus is referred to as multiplex PCR (mPCR). Example, mPCR assay for thesimultaneous detection of *Salmonella*spp., *Escherichia coli* O157:H7, *Listeria monocytogenes,* and*Staphylococcus aureus,* in one tube using specific primers Its, Stx2A, Hly., and Cap8A-B, respectively.

Quantitative PCR (qPCR): also called real-time PCR, is an system competent of continuously monitoring the formation of PCR amplification products throughout the reaction. It offers simultaneous, rapid amplification and sequence-specific-based detection of genes of interest and is progressively being applied in food microbiology.

b)Fluorescent In Situ Hybridization(FISH):

FISH with oligonucleotide probes targeted atrRNA is the most common method among molecular techniques, not based on PCR.The probes used in FISH are generally 15–25 nucleotides in length, and are covalentlylabeled with fluorescent labels or radiolabels (^{32}P). After hybridization, the specificallystained cells are detected using epifluorescence microscopy or autoradiography. FISH in combination with flow cytometry has been employed for rapid culture-independent detection of *Salmonella* spp. on the surfaces of fresh produce like tomato.

c)Isothermal Amplification:

In the recent decade, many novelmethods have been developed to amplify nucleic acidsunder isothermal conditions. These methods include nucleic acid sequence-based amplification (NASBA),loopmediatedisothermal amplification (LAMP), strand displacement amplification (SDA) and rolling circle amplification (RCA). Isothermal amplification has simpler cost-effective hardwarerequirements compared to PCR. Isothermal amplification techniques have displayed superior tolerance than PCR to some inhibitory materials that affectthe molecular amplification efficiency.

4.1.3 Antibody-Based Detection

Immunological detection based on the concept of antigen-antibodybinding is widely used for determining foodborne pathogens. A range of antibodies havebeen used in different assay types for the detection of microbial toxins and foodborne pathogens.

a) Enzyme-Linked Immunosorbent Assay (ELISA):

One of the most commonly used immunological assays forwhich is a very accurate and sensitivemethod for detecting antigens or haptens. Binding of antigen (pathogens or toxins) to the primary antibody is measured quantitatively in a 96-well microtiter plate by using a secondary antibody conjugated to an enzyme. The mostpowerful ELISA format is called the "sandwich" assay,because the antigen to bemeasured is bound between two primary antibodies: thecapture antibody and the detectionantibody. BIOLINESalmonella ELISA test for *Salmonella* spp.,is a rapid, easy, and convenient assay for the detection ofSalmonella in foods and feeds.

b) Immunomagnetic Separation (IMS)Assay:

This procedure which utilizes immunomagnetic beads (IMBs) as capturing reagentsis designed for microbial isolation and identification.IMS is analogous to selective cultural enrichment, in which the growth of other pathogen is suppressed while thetarget pathogen is enhanced. The separation is a two step process,first, the target cells are combined with immunomagnetic particles, incubated (<1h) and separated by asuitable magneticseparator; second, the magnetic complex is washed repeatedly to remove the contaminants. In addition, the IMB surface can be conjugated with bioreactive molecules for the immunoprecipitation, isolation,and identification of biomolecules.

c)Lateral Flow Immunoassay:

Lateral flow assays are a type of immunoassay where the test sample flows along the solid substrate via capillaryaction. After the sample is applied to the test, it encountersa colored reagent (antigen or antibody labeled by gold particles or colloidalor gold particles), which mixes with the sample andtransits the substrate, in the process encountering lines or zones that havebeen pretreated with an antigen or antibody. Depending on the type ofanalytes present in the sample, the colored reagent canbecome bound at the test line or zone. Lateral flow immunoassays such as immunochromatography, dipstick, and immunofiltration are garnering attention in the detection of pathogens and toxins in food

Examples include, immunoassay-basedlateral flow dipstick for the rapid detection of aflatoxin B1 inpig feed. Colloidal immunochromatographicstrip for the detection of *Escherichia coli* O157:H7 inenriched samples.

4.1.4 Biosensor-Based Methods

Biosensor is an analytical device thatcomprises of two main elements: a bioreceptor and transducer. The bioreceptorthat recognizes the target analyte can either be a, biological material (antibodies, enzymes, cell receptor and nucleic acids and cell receptors) or biologically derived material (recombinant antibodies and aptamers) or biomimic (imprinted polymers and synthetic catalysts). The transducer which converts the biological interactions into a measurable electrical signal can be electrochemical, optical, thermometric, mass-based, micromechanical or magnetic

a) Optical biosensors:

Uses Surface Plasmon Resonance (SPR). In SPR, the electromagnetic radiation of a certain wavelength interacts with the electron cloud of the thin metal and produces a strong resonance. When the pathogen binds to bioreceptors which are immobilized on the surface of a thin metal surface, the refractive index changes which results in the change of wavelength required for electron resonance

b) Electrochemical biosensors:

Electrochemical biosensors measure changes in current, impedance, voltage and conductance caused by antigen-bioreceptor interaction and are classified as amperometric, impedimetric, potentiometric, and conductometric, respectively.

c) Mass-based biosensors:

Uses piezoelectric crystals. The binding of the target antigens to the antibodies immobilized on the crystal, this will cause a measurable change in the vibrational frequency of the crystal which correlates with the added mass on the crystal surface.

The rapid techniques for the detection of foodborne bacterial pathogens are summarized in Fig 2.

4.2 Recent developments in detection techniques

Biosensors based on nanofabrication techniques are emerging as rapid-detection methods for food-borne pathogens. Quantumdots are being explored as potential fluorescent probes for detection. Microcantilever-based biosensor for detection of*V. cholerae* O1are being explored. The commercial gold-coated atomic forcemicroscopy microcantilevers are immobilized with a monoclonal antibody and usedto detect*V. cholerae*O1.Another area where research is being focused is in the development of phage-based assays. Phages can be used for both detection and prevention of food-borne pathogen.

4.3 Advantages and limitations of rapid detection techniques

The rapid methods of pathogen/ toxin detection have the distinct advantages of rapidity, specificity,sensitivity, and less samples over conventional culture-based methods. The advantages are summarized below

1. Results are rapid- reduces both labor and time consumed
2. Tests are very specific and sensitive- allows detection of specific serotype
3. The tests and devices are potable
4. Can test for microorganisms that cannot be cultured
5. Allows for earlier detection and faster treatment

However these techniques are also plagued with certain limitations which include

1. Expensive investment in new lab equipments
2. May require additional technical expertise
3. AOAC International approved rapid methods are mostly designated for preliminary screening- negative results are regarded as definite but positive results are regarded as presumptive and need to be confirmed by standard microbiological methods
4. Potential for abuse- bioterrorism

Conclusion

The field of food microbiology has been rapidly and steadily developing in the past century. While conventional methods to detect foodborne pathogens have primarily relied on culture media the rapid methods rely on advanced molecular techniques. Recent developments have shown that they are promising tools on the horizonin the form of quantum dots, phaged-based

assays, and microcantilever biosensors.Given the broad applicability andthe great potential of such methods, there is still a greatchance for further developments

References

1. Doyle, Michael P., and Robert L. Buchanan, eds. *Food microbiology: fundamentals and frontiers*. American Society for Microbiology Press, 2012.
2. Ray, Bibek, and ArunBhunia. *Fundamental food microbiology*. CRC press, 2007.
3. Jay, James M. *Modern food microbiology*. Springer Science & Business Media, 2012.
4. Zhao, Xihong, et al. "Advances in rapid detection methods for foodborne pathogens." *J. Microbiol. Biotechnol* 24.3 (2014): 297-312.
5. Law, Jodi Woan-Fei, et al. "Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations." *Frontiers in microbiology* 5 (2015): 770.

Table1. Major historical developments in food microbiology

Year	Development
1880s	Robert Koch and his associates introduced - solidmedia (first gelatin, then agar) to purify and enumerate bacteria, Petri dish, flagellar staining, steam sterilization of media.
1820	Justin Kerner described food poisoning from eating blood sausage (due to botulism).
1822	C.J. Person- named the microscopic organism found on the surface of wine during vinegar production as *Mycodermamesentericum*which was later renamed byPasteur in 1868 as*Mycodermaaceti* and by MartinusBeijerinck(1898) as *Acetobacteraceti.*
1837	Theodor Schwann named the organism involved in sugar fermentation as Saccharomyces(sugar fungus).
1854	In 1854, FilippoFacini named the cholera bacilli as *Vibrio cholera*, which was isolated in pure form by Robert Koch in 1884
1860	Louis Pasteur showed that fermentation of lactic acid and alcohol from sugar was the result of growth of specific bacteria and yeasts, respectively
1895	Marie von Ermengem isolated *Bacillus botulinus* (*Clostridium botulinum*) from contaminated meat and proved that it caused botulism
1990-2000	Fermented foods-widely commercialized Cutting edge preservation and packaging techniques developed Bacterial etiology of food poisoning established Molecular/ genetic understanding of food borne disease

Source: Ref 2

Table 2. Organisms and metabolites that highly correlate with product quality

Indicators	**Product**
Organisms	
Bacillus spp.	Bread dough
Clostridium spp.	Cheese
Lactic acid bacteria	Wine, beer
Acetobacter spp.	Fresh cider
Byssochlamys spp.	Canned fruits
Zygosaccharomycesbailii	Salad dressing, Mayonnaise
Yeast	Fruit juice concentrates
Metabolites	
Ethanol	Fishery products, apple juice
Volatile fatty acids	Butter, cream
Diacetyl	Frozen juice concentrate
Histamine	Canned Tuna
Lactic acid	Canned vegetable

Source: Ref 1

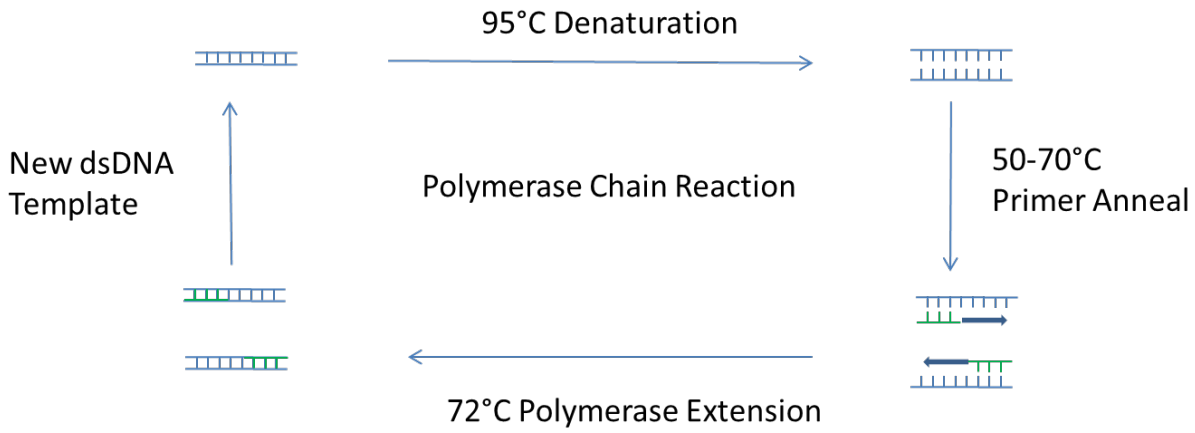

Fig 1. Polymerase Chain Reaction (PCR)

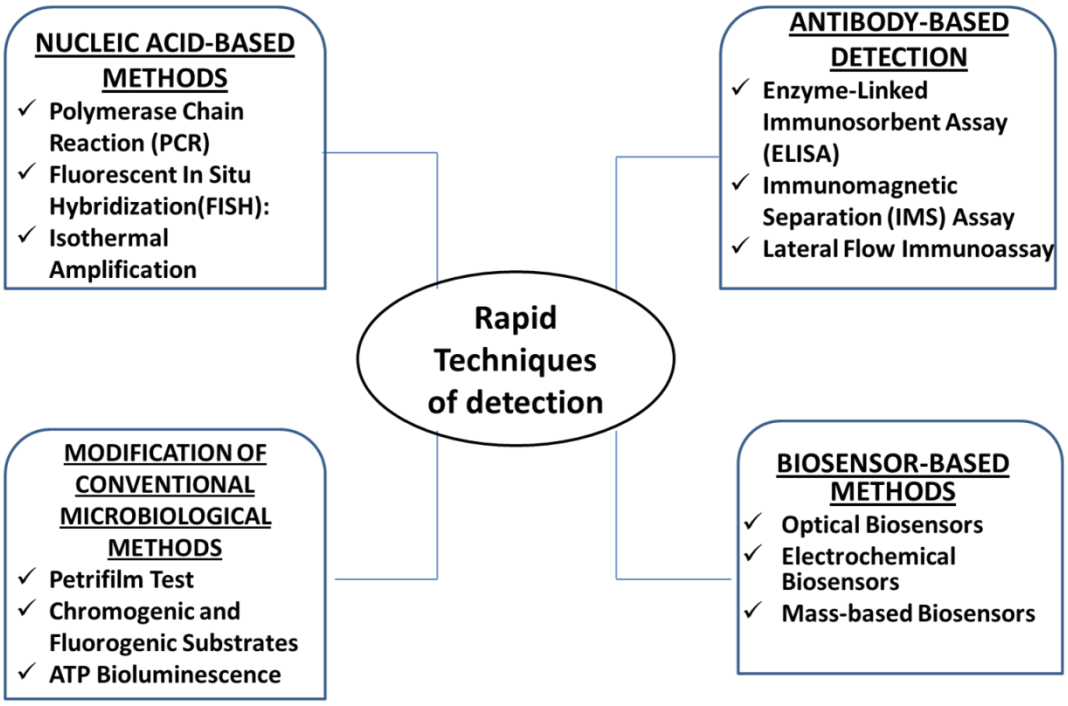

Fig 2.Rapid techniques for the detection of foodborne bacterial pathogens.