Current scenario of pathogen-detection techniques in agro-food sector

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Supplementary Data

Comparative analysis and future perspectives

A comparison of all discussed techniques is provided in Table 1 with their brief description, advantages, and disadvantages [1–8]. The conventional techniques have their own advantages depending on the type of pathogenic targets and the ease of use. Culture plating is old, but it is still being used for ease of use and visual growth confirmation on selective culture media. Techniques such as MALDI-TOF are more refined and use ribosomal protein fingerprinting for more accurate detection [9], whereas the DNA microarray is a more advanced and sophisticated technique that employs a multiplex approach to detect and differentiate samples of various bacteria on the basis of species, subspecies, and functional and metabolic profiles. The multiplex approach is fascinating, but there is much to do in sample processing before multiplex detection. Techniques that used synthetic receptors (such as MIP) are promising to eliminate the use of antibodies as receptors [10]. The integration of omics and CRISPR technology, where omics studies inform CRISPR-based genome editing, enables the engineering of microorganisms and microbiomes' formulation in food [11]. Integrated biosensors are some of the most advanced approaches for the detection of pathogens. The mass production of electrochemical/optical chips using POC settings can be revolutionary to detect various pathogens [12,13].

Table S1. Comparative analysis of different techniques in pathogen detection.

S. no.	Technique	Description	Advantages	Disadvantages	Reference
1	Culture plating and colony counting	Bacterial culture is grown on nutrient medium and their colony-forming units are counted	The most established and relatively easy and cheap method	Requires huge amount of time (16 to 48 hrs) to culture bacteria, prone to contamination, and less specific	[1,14,15]
2	Chromogenic media	Chromogenic bacterial growth occurs due to presence of chromogen in media, which are only catalysed by the specific	Specific for a particular pathogenic stain and	Requires more time and not completely specific	[2,16]

		enzyme present in the pathogenic bacteria	naked-eye detection		
3	PCR	Multiple copies of target DNA are generated	Highly specific and less time consuming	Lack of differentiation between viable and non-viable cells	[3,17–20]
4	Optical biosensors	Optical change is measured when bacterial cell binds to the receptor on the transducer surface	High accuracy, rapid detection and analysis	These optical analyzers are expensive and require expert handling	[21–27]
5	MALDI-TOF	Pathogen-specific peptide mass fingerprint (PMF) is used to identify the pathogen in the given sample after treating the sample in MALDI-tof mass analyzer.	Complements the conventional culture method	The mass spectrometry is expensive and requires expert handling	[4,9,28,29]
6	Paper-based and lateral flow assay	Chromogenic reaction on paper is due to bacterial enzyme or receptor-cell binding and lateral flow of the analyte and its detection due to enzyme-linked chromogenic receptor	Takes less time and is cost effective	Poor detection limits	[5,30,31]
7	Molecular imprinting	Pathogenic capture polymer surfaces are generated using molecular imprinting to select and bind bacteria	Replaces antibody, rapid, and cost effective	Requires generation of molecularly imprinted polymers	[10,32,33]
8	Electrochemical collusion technology	Bacterial detection is done by measuring the change in diffusion	Very sensitive and rapid	Requires the electrochemical station	[34,35]

		current caused by the collusion of cell with the ultramicroelectrode surface			
9	DNA microarray	Simultaneous identification and detection of different bacterial cells by is done by cDNA hybridization of specific genes associated with specific bacterial cells	Identifies different types of bacteria in one reaction	Use of expensive microarray plates and reagents and also requires expert handling	[6,36,37]
10	Antibody-based immunoassay	Target analyte is detected on the basis of antibody-antigen reaction in the presence of enzyme	Specific and possible handling of large number of samples	Low sensitivity and issue of cross-reactivity with closely related antigens	[38,39]
10	Aptamer-based immunoassays	Various immunoassays are developed using synthetic DNA/RNA receptors	Replaces antibody and is highly sensitive	Requires generation of target-specific aptamers	[40–42]
11	Omics-based technologies	These techniques (such as next-generation sequencing, proteomic, etc.) study changes in protein, mRNA, and DNA under different physiological and environmental conditions	Early detection of wide range of bacterial strains	Complicated isolation of components in a complex system	[7,43]
12	CRISPR-based technologies	This technique involves the cleavage of target nucleic acid via endonuclease activity associated with CRISPR-Cas enzyme	Specificity, ease-of-use, and accuracy	Need for complex assays in case of preamplification, which is required for detection of	[7,44]

				targets below fmol range	
13	Electrochemical assays	Various electrochemical techniques are used for detection	Rapid and sensitive detection	Requires expensive electrochemical station	[35,41]
14	Nanomaterial-mediated detection	Unique optical and electrical properties of nanomaterials are utilized for the detection of bacterial cells	Rapid and low-cost detection	Requires generation of nano-bioconjugates, and these conjugates have stability issues	[8,45–48]

CRISPR: Clustered regularly interspaced short palindromic repeats
DNA: Deoxyribonucleic acid
MALDI-TOF: Matrix-assisted laser desorption-time of flight
PCR: Polymerase chain reaction
RNA: Ribonucleic acid

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