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Review

Biosensors as innovative tools for the detection of food borne pathogens

Pooja Arora, Annu Sindhu, Neeraj Dilbaghi, Ashok Chaudhury*

Department of Bio and Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar 125001 (Haryana), India

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ABSTRACT

The wholesomeness of food is the real proviso for healthy life. Food freed from microbial and chemical cross-contaminations adds on to its hygienic and nutritive value. Infectious diseases spreading every day through food have become a life-threatening problem for millions of people around the world. Food or food products are the potent transmitting agent of more than 250 known diseases. So far only in the United States, 76 million cases of food-borne illness, 32,500 cases of hospitalization and 5000 cases per annum of mortality are recognized. Health expert's estimate that the yearly cost of all the food borne diseases is approximately $5–6 billion. There is therefore, is an urgent need for the development of rapid, competent, and reliable methods for direct detection and identification of foodborne brown pathogens. In this overview, we have concentrated specifically on microbe-based biosensing methods such as optical, surface plasmon resonance (SPR), amperometric, potentiometric, whole-cell, electrochemical, impedimetric, piezoelectric for the rapid detection of food borne pathogens. Furthermore, we have focused our attention on the discussion of principal concepts, applications, and examples from analyte to the configuration of potential biosensors that have been achieved up until now to detect potential foodborne pathogens. The article presents foreseeable future trends in biosensor research activities for paving the way for fresh and healthy food proposal.

Contents

1. Introduction

Nanotechnology composes a trend towards a group of emerging techniques from physics and biology for the creation of new fangled nanostructures and manipulation of the matter at nanoscale

* Corresponding author. Tel.: +91 1662 263165; fax: +91 1662 276240.
E-mail address: ashokchaudhury@hotmail.com (A. Chaudhury).

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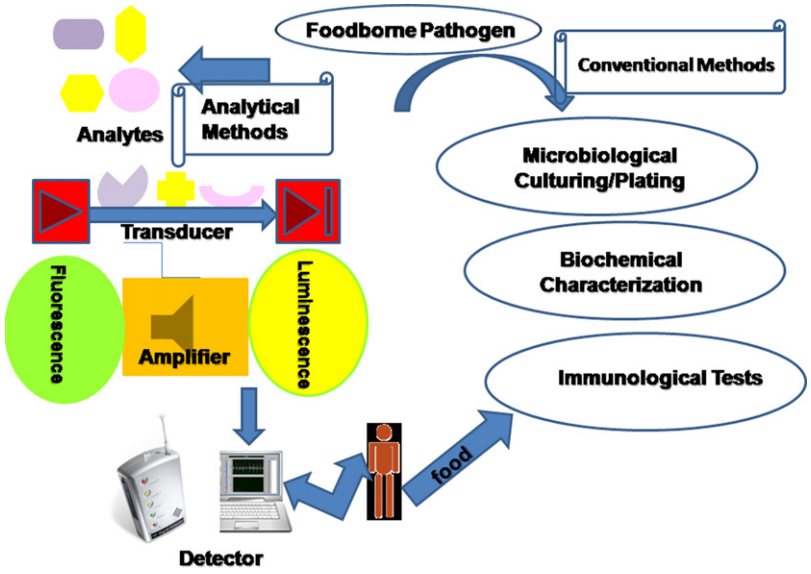

Fig. 1. The analyte–receptor binded modified transducer approach as a cutting edge over conventional protocols for the detection of food borne pathogens.

(Kossek et al., 1998). This novel technology is now concentrating on *in vivo* sensors (nano-sized devices), so that on being injected it could act as reporters of *in vivo* concentrations of chief analytes (LaVan et al., 2003). Biosensors are categorized into various groups according to the basic principles of signal transduction and biorecognition elements. According to the transducing elements, biosensors can be classified as electrochemical, optical, piezoelectric, and thermal sensors. Applications of biosensors are developed majorly for environmental and bioprocess monitoring, quality control of food, agriculture, bioterrorism and medical biosensor systems. Food and beverage industry requires reliable analytical methods for the determination of specific components such as sugars, proteins, vitamins, and fats for the detection and quantification of chemical contaminants such as pesticides, heavy metals, antibiotics, and pathogenic microorganisms. The recent attention to food safety and regulatory issues towards consumer welfare is of utmost concern. Analytical techniques such as spectrophotometry and chromatography are time-consuming and expensive. They often require well trained operators for the sample pre-treatment steps, and cost-effective analysis. Biosensors offer advantages over current analytical methods. Besides their good selectivity and low cost, they are portable to use in working sites and have the ability of measuring samples with minimal sample preparation. In fact, for the availability of fresh products in the food industry, biosensor research has been focused on the contaminant detection, content verification, monitoring of raw materials conversion and product freshness (Collings and Caruso, 1997). The beer industry has already carried out the use of biosensors for identifying the ways to improve and control their commercial product goods (Meadows, 1996).

In the past decade, so many review papers have been published to address the development of microbial biosensors (Vikesl and Wigginton, 2010). Sua et al. (2011) has recently reported one such collective finding on microbial biosensors. For this reason, a comprehensive literature survey has been carried out with emphasis on the overview in the field of food borne pathogen detection. This review article tends to discuss the bottleneck achievements in the fabrication and application of specifically food borne pathogenic biosensors. Future prospective for the detection of food pathogenic biosensors will also be discussed.

2. Biosensor

Due to the green revolution in the past decade, concern for food safety as well as quality has gained importance. Nevertheless, the likelihood of contaminated food has also increased by foodborne pathogens and toxins. A leading cause of contamination worldwide is microbial toxins along with agrochemicals. Any pathogenic microorganism in food can lead to severe health consequences in animals and humans. Most prominent food borne pathogen includes mycotoxins, exotoxins and enterotoxins from *Escherichia coli* O157:H7, some strains of *Staphylococcus aureus*, *Shigella* spp., *Bacillus anthracis* (produces anthrax toxin), *Campylobacter jejuni*, *Clostridium perfringens*, *Clostridium botulinum* (produces a powerful paralytic toxin botulin), *Salmonella* spp., *Listeria monocytogenes*, *Vibrio cholera*, *Yersinia enterocolitica* and> *Coxiella burnetii* (Feng, 2001; Moss and Adams, 2008a,b). Risk group includes babies, young children, pregnant women (and their foetuses); elderly and sick people and others with weak immune systems, which gets highly affected by mycotoxin outbreaks (Feng, 2001; Moss and Adams, 2008a,b). Vegetativly, conventional techniques such as polymerase chain reaction (PCR) (Burtscher and Wuertz, 2003), culture and colony counting (Allen et al., 2004), immunological assays (Van et al., 2001) and fluorescence spectrophotometry using organic dye molecules (Regnault et al., 2000) are the budding approaches for research observations. The studies have found several drawbacks associated with conventional methods over analytical methods for detection of fatal diseases. Fig. 1 explains that the analyte-receptor binded modified transducer approach has a cutting edge over conventional protocols for the detection of food borne pathogens. These techniques are time-consuming as well as laborious and must be performed by skilled personnel only. Moreover, the results are not accurate in most of the cases. Culture methods are susceptible to contamination, and organic fluorescent dyes to photo blanching effects can lead to spectral overlaps that make it inappropriate for multiplex detection. To overcome these limitations, an immediate need for the development of some edge alternative succeeded with an analytical approach. A biosensor-based process is robust state-of-art for broad practical applications in diverse pathogenic bacteria detection. A modified and updated list of sensors to detect food borne pathogens is addressed in Table 1 and Fig. 2. The emergence of

Table 1
Sensors to detect various food borne pathogens (adapted source: Pedrero et al., 2009).

Methodology	Analytes	Sample	Analysis time	Detection limit	Reference
Cell based sensor	*Listeria monocytogenes*	Food	–	–	http://www.cfse.purdue.edu/media/annualreport/panel_left.pdf
Acetyl-choline-esterase (AChE) based inhibitor biosensor	Organophosphate pesticides	–	–	–	Guodong and Yuehe (2006)
CNT DNA linked biosensor	*Salmonella typhi*	–	–	–	http://singularityhub.com/2009/09/22/new-biosensor-finds-bacteria-in-seconds/
Impedance-based fieldable immunosensor	*E.* coli O157:H7 *Salmonella* spp.	–	Response time <1 min	10 CFU *E. coli* O157:H7	Louie et al. (1998)
Impedance-based HRP labelled immunosensor	Rat IgG, Hepatitis B virus surface antigen (HBsAg), Hepatitis B e-antigen (HBeAg)	–	–	10 pg $mL^{-}1$ HBsAg	Yu et al. (2006)
Poly di-acetylene (PDA) using a novel immobilization procedure	–	–	–	–	http://web.skku.edu/nmndl/publications/26%20-%20AFM.pdf
Magnetic	*Influenza A adenovirus*, *Mycobacterium avium* spp., *Paratuberculosis*, *E. coli* O157:H7	–	–	single virus detection (PBS) 15.5 CFU (milk) 10^4 CFU/mL (TWEEN/PBS)	Patolsky et al. (2004) and Kaittanis et al. (2007)
Ag-Potentiometric stripping analysis (PSA)-based DNA Sensors	*Stachybotrys chartarum*, Escherichia coli	–	–	2.0×10^{-12} M oligonucleotides	Cai et al. (2004)
IDA-AP amplification based RNA microarray sensors	*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*	–	Fully automated detection in less than 25 min	0.5 ng μL^{-1} (16 fmol) *E. coli* RNA	Elsholz et al. (2006)
IDA-β-Gal amplification based DNA microarray sensors	*Bacillus cereus*	–	–	–	Liu et al. (2008)
IDA-AP amplification based DNA microarray sensors	*Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis* and *ortho pox viruses*	–	Fully automated detection in less than 27 min	–	Elsholz et al. (2009)
SPE-AP amplification based array DNA sensors	*Listeria monocytogenes* toxin		Total analysis time < 1 h	0.75 nM	Laschi et al. (2006)
HRP-amplification-based DNA multiwell sensor strips	*Escherichia coli* O157:H7, *Salmonella* spp., *Campylobacter jejuni*, and *Staphylococcus aureus*	Natural beach water spiked with human faeces, and water and sediments collected from New Orleans (LA, USA) following Hurricane Katrina	3–5 h	$\leq$ 1000 cells *Karenia brevis*	LaGier et al. (2007)
Chrono coulimetric respiratory cycle activity measurements and PCA chemometric data treatment-based methodology	*Baccilus cereus*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Enterobacter aerogenes*, *and Saccharomyces cerevisiae*	–	–	–	Ertl and Mikkelsen (2001)

Table 1 (*Continued*)

Methodology	Analytes	Sample	Analysis time	Detection limit	Reference
Esterase 2-amplification based DNA array sensor	*Escherichia coli*, *Bacillus subtilis*, *Bacillus atrophaeus*, and *Listeria innocua*	Meat juice	One working day	500 CFU *E. coli*	Pöhlman et al. (2009)
Hoechst 33258-based DNA array sensor	*Clostridium piliforme*, *Helicobacter bilis*, *Helicobacter hepaticus*, and mouse hepatitis virus	Mice caecum, faeces, heart and liver	–	10^{-2} CFU *C. piliforme*	Goto et al. (2007)
HRP-amplification-based DNA microarray sensor	*Bordetellapertussis*, *Streptococcus pyogenes*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*	–	–	2 fg *M. pneumoniae*	Lodes et al. (2007)
Potentiometric aptamer-based biosensor	*E. coli*	Apple juice, milk		6 colony forming units/mL (CFU) in complex matrices such as milk or 26 CFU/mL in apple juice	Gustavo et al. (2010)
HRP-amplification-based DNA microarray sensor	*Bacillus anthracis*, *Yersinia pestis*, *Escherichia coli and Bacillus subtilis*	–	–	0.75 pM	Ghindilis et al. (2007)
Ag-PSA-based DNA sensors	*Escherichia coli Bacillus subtilis*	–	–	–	Yeung et al. (2006)
Piezoelectric-excited millimeter-sized cantilever (PEMC) biosensor	*Giardia lamblia*		15 min		Xu and Mutharasan (2010)
SPE-AP amplification based array DNA sensors	*Salmonella* spp., *Lysteria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*	–	Total analysis time < 1 h	–	Farabullini et al. (2007)
Chrono coulimetric respiratory cycle activity measurements and PCA chemometric data treatment-based methodology	*Escherichia coli B*, *Escherichia coli Neotype*, *Escherichia coli* JM105 *and E.coli* HB101	–	Total analysis time 40 min	–	Ertl and Mikkelsen (2001)
Microscale impedance based metabolic activity detection-based methodology	*Listeria innocua*, *L. monocytogenes*, *Escherichia coli*	–	2 h	100 *L. innocua*, 200 *L. monocytogenes*, and 40 *E. coli* cells	Gómez et al. (2002)
Electrochemical oxygen multisensor array and PCA chemometric data treatment-based methodology	*Corynebacterium glutamicum*, *Micrococcus luteus*, *Staphylococcus epidermis*, *Yersinia ruckeri*, *Escherichia adecarboxylata*, *Comamonas acidovorans*, *Bacillus globigii*, *and three strains of Escherichia coli*	–	–	–	Karasinski et al. (2005)
Electrochemical oxygen multisensor array and PCA chemometric data treatment-based methodology	*Escherichia coli*, *Escherichia adecarboxylata*, *Comamonas acidovorans*, *Corynebacterium glutamicum* and *Staphylococcus epidermidis*	–	8 h	1×10^6 CFU mL^{-1}	Karasinski et al. (2007)

Table 1 (*Continued*)

Methodology	Analytes	Sample	Analysis time	Detection limit	Reference
Electrochemical oxygen dual sensor	*Ralstonia solanacearum*, *F. oxysporum* f. sp. *Lactucae* strain, *Plasmodiophora brassicae*, and *F. Oxysporum* f. sp. *spinaciae*	Soil	–	–	Hashimoto et al. (2008)

nanoscience and nanotechnology simulate the scientists to fabricate devices used in bio analysis (via integration of nanomaterial with bio molecules). These bio recognition devices are capable of rapid (in few minutes) and sensitive detection (even of single cell) of food borne pathogens. Polymeric nanoparticles (Yang et al., 2007a), liposomes (Ho and Hsu, 2003; Chen et al., 2005; Chen and Durst, 2006), vesicles, inorganic semiconducting, metallic and magnetic nanoparticles were conjugated with bio molecules such as antibodies, antibiotics, adhesion molecules and complementary DNA sequences for specific recognition of pathogens. Miniaturization of devices has created lab on small chips, which enables detection of pathogens by portable, hand held biosensors (Nugen and Baeumner, 2008). Versatile chemistry, unique optical properties and strong ferromagnetic responses (Lin et al., 2002; Gu et al., 2003; Naja et al., 2007; Yang et al., 2007b) have made nanoparticle as an efficient tool for various bio detector systems. Subsequently, nanomaterial based genome and proteome detections had been used for several significant advancements (Rosi and Mirkin, 2003; Zhang et al., 2007). Development of nanoscale materials such as nanowires, nanofibers, nanoparticles, nanobelts or nanoribbons and nanotubes has revolutionized clinical and molecular biology by their significant use as bioanalysors and biodetectors and now the days it is widely used for figuring out the contamination in food materials. The objective of rapid and sensitive detection of food borne infections is successfully achieved by using nanoparticles in very low doses (Yang et al., 2008). It is easier to detect multiple pathogens at a single time from liquid and solid food products through real-time bio-functionalized devices. The diverse capabilities of nanomaterials have made significant contributions in bio imaging, biosensing, drug delivery and design of multifunctional nanodevices (Chan, 2006). Several label free biosensors are recently in use for food borne pathogen detection (Lazcka et al., 2007; Bhunia, 2008; Zourob et al., 2008). These make use of surface plasmon resonance, amperometric and potentiometric

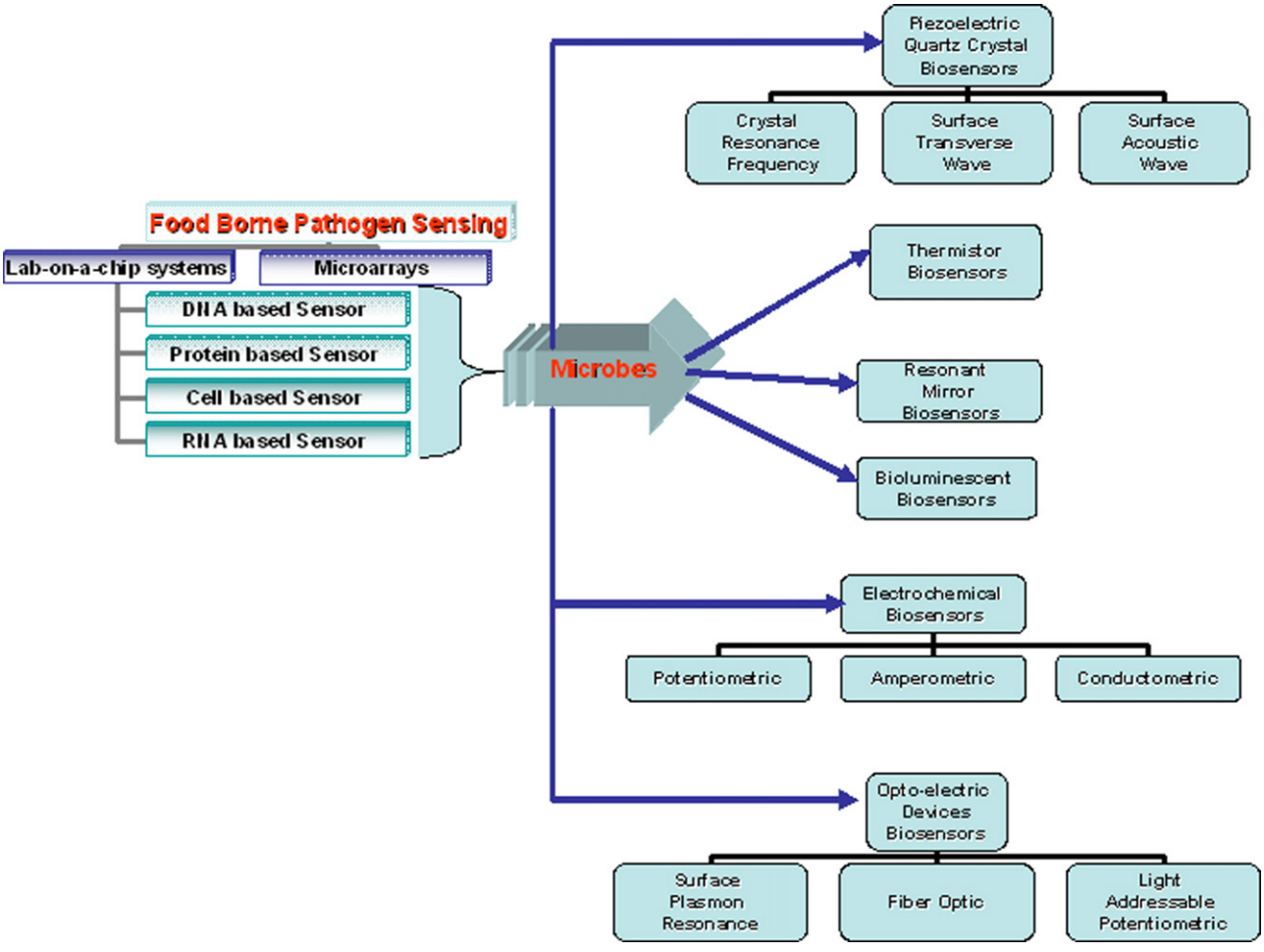

Fig. 2. Schematic illustration of biosensor technologies to detect food borne pathogens at glance.

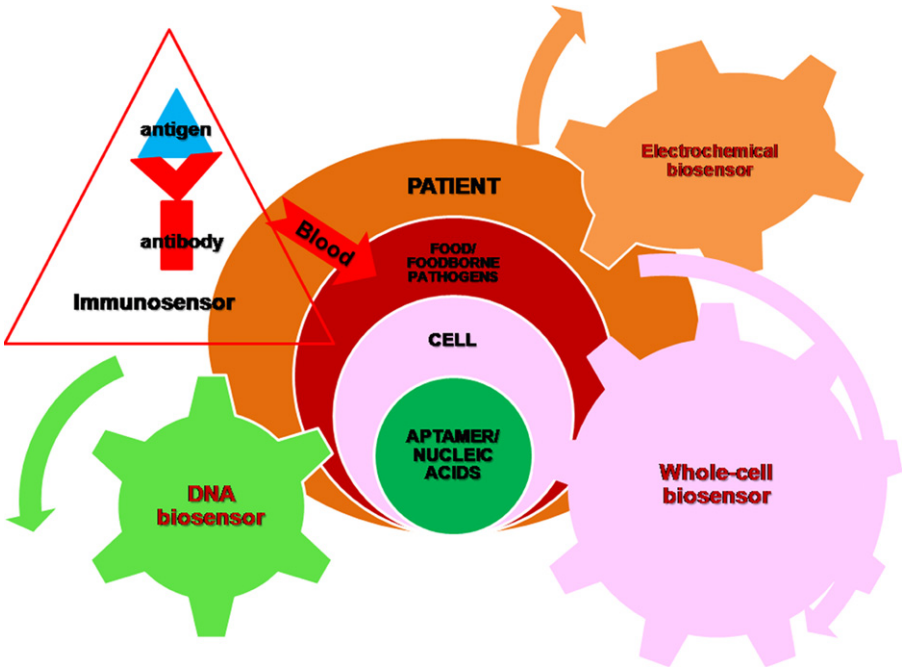

Fig. 3. Multiple biosensor configurations for pathogen detection.

measurements or electrochemical impedance spectroscopy instead of fluorescent labels for detection purposes, which were based on biological recognition elements such as enzymes, antibodies and nucleic acids. The modality utilizes spectroscopic (Raman and IR) (Helm et al., 1991; Naumann et al., 1991; Rosch et al., 2003; Harz et al., 2009; Willemse-Erix et al., 2009), autofluorescence (Estes et al., 2003; Ammor et al., 2007), MALDI-TOF (Lay, 2001; Dare, 2006; Seng et al., 2009), and light-scattering (Wyatt, 1969; Wyatt and Phillips, 1972; Steen, 1990; Rajwa et al., 2008) based systems for bacterial detection. The light scattering detection method completely relies on quantitative description of optical properties of the microorganisms therefore; it is superior for vibrational spectroscopic methods. Rapid detection of pathogenic food bacteria can be carried out through recently developed optical sensors. These sensors work on light scattering technology and can be efficiently used for detection as well as identification purpose of food borne pathogens because of characteristic light pattern generated by scattering of incident laser light; through different strains of bacteria. Differentiation even up to genus, species and strain level was carried out for bacterial colonies such as *Listeria*, *Staphylococcus*, *Salmonella*, *Vibrio*, and *E. coli* (Bayraktar et al., 2006; Banada et al., 2007, 2009). It relies on forward-scatter phenotyping (FSP) and able to classify bacterial colonies in a non-exhaustive framework. These optical biosensors also act as a promising technique for automated detection of unknown bacterial colonies (Rajwa et al., 2010).

3. Biosensor configurations

Traditional methods of detection are very sensitive and inexpensive but equally they are too laborious, time-consuming, and merely depend on the properties of bacteria to form frequent clones (Biswas, 2005). One needs to proceed from pre-enrichment, selective plating, biochemical screening as well as/till serological confirmation of the results (Vunrcrzant and Pllustoesser, 1987). From avoiding, the havoc resulted from food borne pathogens there is a need to get immediate information about the possible presence of pathogens in raw material as well as in finished products. A single pathogenic organism in food can be at an infectious dose. Biosensors act as an indicator of biological molecules with the required features of on spot and multiple analyses systems (Fig. 3 supports multiple biosensor configurations for pathogen detection). These rely on nucleic acid analysis, metabolic patterns of substrate utilization by a bacterium, analysis of pathogen interaction with eukaryotic cells, and analysis of signature molecules by antibodies. By exploiting the various physiological and genetic properties of microorganisms present in foodstuffs, different biosensors were proposed and fabricated with robust validity, speed, and automation. A schematic list of applied and commercialized transducers is shown in Table 2.

3.1. Optical biosensor

Selectivity and sensitivity of optical biosensors have put them in the category of most popular biosensors for detection purposes. Amongst optical biosensors, fibre optic is the first commercially available sensor marketed by Research International (Monroe, WA). The basic principle of fibre optic biosensor lies behind fluorescently labelled pathogens or toxins, which when bound to the surface of biosensor gets an excitation by the laser wave (635 nm). Consequently, it generates fluorescent signals (Bhunia, 2007; Taitt et al., 2005) which were detected by fluorescent detector in the real time system. Various optical biosensors for rapid detection of pathogenic bacteria (Baeumner et al., 2003), toxins (Bae et al., 2004) and contaminants (Willardson et al., 1998; Tschmelak et al., 2004) in food were developed. In fluorescent biosensors, a fluorescent compound in conjugation with antibodies aids in the detection of pathogenic bacterium. Most routinely used fluorescent marker is FITC (fluorescein isothiocyanate) (Li et al., 2004), though some lanthanides are also considered for the same purpose (Selvin, 2002). Additionally, it can also be used comfortably in PCR and ELISA for detection. Higgins et al. (2003) have developed a hand held real-time thermal cycler for enabling the detection of several bacteria simultaneously within the detection limit of 30 min. Now a day's various antibody-coupled fibre-optic biosensors are being developed for the detection of botulinum toxin, staphylococcal enterotoxin, *E. coli* O157:H7, Listeria, and *Salmonella*.

Table 2
Schematic list of applied and commercialized transducers.

Transducers	Bio-recognition elements	Measured property	Company
Electrochemical	Enzymes, proteins, amino acids nucleic acids: DNA, RNA, PNA, antibodies, antigens, specific genes, organelles, microbial cells, plant and animal tissues	Potentiometric, conductometric, field effect	Malthus 2000 (Malthus Inc., Stoke-on-Trent, UK)
		Amperometric	Midas Pro (Biosensori SpA, Milan, Italy)
		Voltametric	
Electrical		Impedimetric	Bactometer (Bactomatic Inc., Princeton, USA)
		Surface plasmon resonance	Biosensing Instruments, IBIS Technologies, Bio-Rad, Reichert, iCx Technologies, GWC Technologies, Sensata Technologies
		Surface conductivity	BIA-core (Pharmacia, Uppsala, Sweden), GE Healthcare
		Electrolyte conductivity	
Optical		UV Absorption, fluorescence emission, optical quantitative imaging	Nanolane
		Adsorption, bioluminescence, chemiluminescence	Lumac Biocounter (Lumac B.V., Schesberg, Netherlands)
		Optical grating coupler sensing technology	OWLS Sensor
Mass sensitive		Reflection, internal reflection spectroscopy, laser light scattering	Unilite (Biotrace, Bridgend, UK)
		Grating based light diffraction	Axela
Thermal		Resonance frequency of piezocrytals, piezoelectric	PZ 106 Immuno-biosensor System (Universal Sensors, New Orleans, USA)
		Heat of reaction, Heat of adsorption	MicroCal/GE Healthcare
		Thermistor sensor	Thermal activity monitor (Thermometric, Northwich, Cheshire, UK)
		Acoustic wave modes	
		Quartz crystal	Attana sensor technologies, KSC Instruments, QSENSE
		Cantilever sensor technology	Concentris, Cantion/Nanonord
		Imaging ellipsometry	Accurion/Nanofilm
		96-Well label free detection	CORNING
		Bio-layer interferometry	ForteBio

3.2. Surface plasmon resonance biosensor

Optical illumination of metal surface is one amongst the target sources for food borne pathogen detection. Surface plasmon resonance (SPR) sensor works on this phenomenon. Antibodies were initially immobilized on thin gold film just over the reflecting surface of waveguide, to capture various pathogens. At certain wavelengths and near IR region, strong resonance was generated through the interaction of light with the electron cloud in the metal. Binding of pathogen to the metal surface causes a shift in resonance to longer wavelengths and amount of shift accordingly reflects the concentration of bound pathogen or toxins. SPR biosensors can detect molecules even in femtomolar range (Bhunia, 2007; Rasooly and Herold, 2006). Although this optical based SPR system has been used for detection of whole cells of *E. coli* O157:H7, *Salmonella*, and *Listeria* at traceable concentrations, it also showed strong signals with small toxin molecules, such as staphylococcal or botulinum. Literature shows the successful applications of SPR for pathogenic bacteria detection by means of immunoreactions (Taylor et al., 2005; Oh et al., 2005).

3.3. Piezoelectric biosensors

Piezoelectric sensors are another class of biosensors used in pathogenic detection rationale. These mass sensitive detectors generate and transmit acoustic waves with an oscillating crystal, which resonates at the principle of natural resonance frequency. Recently, after quartz, lithium niobate has now been included in the piezoelectric raw material list (Leonard et al., 2003). The surface of piezoelectric sensor is coated with bacterial specific antibodies. The binding of bacteria with antibodies results in increased mass of quartz crystal and proportionate decrease in oscillation frequency, which is detected by the quartz crystal microbalance (QCM) on transducer surface (O'sullivan and Guilbault, 1999). It is very attractive, simple and real time technique for food borne pathogen detection. Babacan et al. (2006) have used probes modified with protein-A antibody for detecting the presence of highly piezoelectric bacterium *Salmonella typhimurium* on the surface of piezoelectric biosensor.

3.4. Cell based sensors

These biosensors also serve as a reliable tool for detection of pathogens in food samples. Cell based assays make use of electrical properties of cells to find out the changes in the cell's vicinity. Cell membrane functions as capacitor while the fluid acts as resistance element. Electrical impedance (EI) senses the changes in cell density, growth, as well as in the diversification of cell normal activities, by the influence of external environment. Mammalian cells are used for the detection of pathogenic potential of food borne diseases (Bhunia and Wampler, 2005; Gray, 2004). Detail information on cellular morphology and growth pattern of tissue culture can also be gained with cell-based biosensors. It is easier to detect enzymes and co-factors from the metabolic system of cells as during the process they potentially release large number of chemical compounds. Additionally, the progress in recombinant DNA technology has opened endless possibilities of tailoring the microorganisms to improve the activity of enzymes or whole cells making microbe an excellent source to consume or degrade the new substrates under certain cultivating conditions.

3.5. Electrochemical sensors

The present popularity of analytical biosensors is due to significant advantages possessed by these biological detectors. These are highly sensitive in miniaturized detectors and can operate well in turbid media. These features allow very less amount of sample free preparation of natural food samples. One of the reasons for their popularity is the simple use of analytical methods and their low economical cost. These sensors are very sensitive and have been used to detect *Salmonella* and *E. coli* O157:H7 in less than 90 min. Depending on the type of transducer used, these are classified as amperometric, potentiometric, conductometric, or impedimetric biosensors. Moreover, the emergence of electrochemical immuno- and DNA platforms has pave the way to distinguish the target pathogens specifically in a multi organism's matrix, the adaptability to detect respective analytes, the sensitivity to detect bacteria online excluding laborious pre-enrichment step, and the robustness to give real-time results.

3.6. Amperometric biosensors

These biosensors are able to detect only the electrochemically active analyte, an analyte capable of being oxidized or reduced on the electrode. Generally, these are prepared with thin-film technology and consist of gold (Au), platinum (Pt) or carbon. Inks in form of thin films in a particular pattern and thickness were deposited on the electrode substrate (glass, plastic or ceramic) for screen-printing. Different inks can be used for getting different dimensions and shape of biosensors. These days screen-printed electrochemical cells are widely used for developing amperometric biosensors because these are cheap and therefore, can be produced at large scale. This could be potentially used as disposable sensor that decreases the chances of contamination and prevents the electrode from fouling which results in loss of sensitivity as well as reproducibility of the biosensor. Amperometric biosensors generally rely on an enzyme system that catalytically converts electrochemically nonactive analytes into electrochemically active products. Horse radish peroxidase (HRP) and alkaline phosphatise (AP) are amongst the commonly used enzymes. These biosensors are used as immunosensors or genosensors for detecting enzyme labelled traces. One major drawback of these biosensors is the generation of false current values by different electroactive compounds leading to potential interferences. This limitation may overcome by use of selective membranes capable of controlling the access of compounds to electrodes based on weight or charge possessed by them. Microbial metabolism-based, antibody-based (immunosensors), and DNA based biosensors, are the potential amperometric biosensors used in food borne pathogen detection.

3.7. Microbial metabolism-based biosensor

Amperometric transducers were used for the measurement of the biochemical reactions taking place during various metabolic processes in bacterial cells. Clark-type oxygen electrode is used for measuring oxygen consumption in the cells (Patel, 2002; Suzuki et al., 1991) subsequently, with the monitoring of highly pathogenic food bacterium *S. typhimurium* in *in situ* conditions. It is carried out by measurement of cathodic peak current of oxygen during bacterial proliferation (Ruan et al., 2002). Another detection approach using electrochemical transducers relies on the detection of specific marker enzyme after incubating in a suitable medium. This strategy aids in easy coliforms detection in water samples by the mere presence of enzyme β-D-glucuronide, glucuronosohydrolase (GUS) and β-D-galactosidase (β-GAL). Conventional methods of *E. coli* detection using GUS enzymes or β-GAL are lengthy and rely on spectrometric detection of bacterium. The bacterial cultures are firstly, incorporated with a chromogenic substrate like p-nitrophenyl-β-D-glucuronide (PNPG) and then being monitored spectrophotometically, until release of chromophore indicators (p-nitrophenol (PNP) and D-glucuronic acid) of GUS reporter, hence confirming the *E. coli* infection in a sample. To over edge this time-consuming protocol, an efficient electrooxidative method for GUS detection using bacteria-based biosensors has been developed (Mulchandani et al., 2005) by immobilization of *Moraxella* species on a carbon paste electrode that degrades PNP and produces hydroquinone (intermediate) for oxidation at a lower potential than PNP. For enumerating coliforms in water media, β-GAL has commonly applied to good yield. It catalyzes the breakdown of lactose into galactose and glucose. A rapid *E. coli* detection method for viable *E. coli* cells were developed by Perez et al. (2001) using enzyme β-D-galactosidase that converts 4-aminophenyl-β-D-galactopyranoside (4-APGal) to 4-aminophenol (4-AP) after hydrolysis. Serra et al. (2005) has developed a novel and improved tyrosinase composite biosensor based on amperometric detection of β-galactosidase activity. The hydrolysis of phenyl-D-galactopyranoside (PG) by β-galactosidase releases phenol as the end product, thus, confirms the success of sensor.

3.8. Immunosensors

Detection with antibodies, which are specific for particular microorganism, or toxin detection is the basic approach utilized in these immunosensors. A list of applications pertaining to detection of microbial contamination in food using immuo-transducers is sequenced in Table 3. Antibodies for this sake can be immobilized on the electrode surface or on magnetic beads that leads to categorization of these immunosensors as: immunosensor based on antibodies immobilized directly on the electrode and immunosensors based on antibodies immobilized on magnetic beads. In immunosensors, enzyme-substrate catalysis in conjugation with an antibody produces products such as ions, pH change, or oxygen consumption, which are capable enough of generating an electrical signal on a transducer. Several approaches were assayed for the biosensor immuno-module operations, including an antibody-based system for the detection of the food borne pathogens with anti-*E. coli* O157:H7 and *Salmonella* spp.

3.9. DNA-based biosensors

In recent years, DNA-based biosensors have tremendously applied for the detection of pathogens. Short nucleic acid sequences called as probe specific for a particular bacterium were immobilized on the surface of a transducer. Complementary binding to the bacterial DNA sequences in the probe sequence does detection of pathogenic bacterium; this event is termed as hybridization. Extent of hybridization determines the presence or absence of complementary sequences in the sample. Some reviewers (Kerman et al., 2004; Palecek, 2002; Palecek and Fojta, 2001; Wang, 2002; Pividori et al., 2000) have cited recent hike in the use of electrical transducer in combination with DNA based detection. Detection of different pathogenic bacterium can be done with disposable low-density genosensor array. It is fabricated using screen-printed array of gold electrodes having immobilized thiol-tethered oligonucletide and biotinylated signalling probes (Farabullini et al., 2007) for the detection of sequence complementarity. Analysis strategy relies on the identification of toxin produced by the specific bacteria and for this sake sequence of gene, encoding strain specific toxin was chosen as the prime criteria for selection. This is the most crucial step of the assay, as the encoded genes; frequently express the toxins in food. Wang (2002) has successfully developed novel genosensors for *Cryptosporidium*, *E. coli*, *Giardia* and *Mycobacterium tuberculosis*. The carbon pasted electrodes and chronopotentiometry are

Table 3
Detection of microbial contamination in food using immuo-transducers.

Etiology	Source	Symptoms	Infection time	Transducer	Biorecognition element	Laboratory testing	Disease
E. coli O157:H7	Lettuce, Carrots, Rucola	Nausea, severe abdominal cramps, watery/very bloody diarrhea, tiredness, fever or vomiting	6–8 days	Potentiometric	Antigen-antibody	Stool culture; *E. coli* O157:H7 requires special media and specific testing; Shiga toxin testing may be done using commercial kits; positive isolates should be forwarded to public health laboratories for confirmation and serotyping	Gastroenteritis, Bacillary dysentry
E. coli O157:H7	Lettuce			Impedometric	Antigen-antibody		
E. coli O157:H7	Lettuce, Alfaalfa, Sprouts, Strawberries			Conductometric	Antigen-antibody		
E. coli O157:H7	Chicken			Amperometric	Antigen-antibody		
E. coli	Water			Amperometric	Microbe metabolism		
E. coli	Water			Amperometric	Microbe metabolism		
E. coli	Water			Amperometric	Microbe metabolism		
E. coli	Water			Amperometric	Aptamer		
Salmonella	Chicken	Diarrhea, Fever, Abdominal cramp, Headache	4–7 days	Potentiometric	Antigen-antibody	Routine stool cultures	Paratyphoid, Typhoid fever
Salmonella	Meat			Amperometric	Antigen-antibody		
Salmonella	Chicken			Amperometric	Antigen-antibody		
Campylobacter jejuni	Chicken	Bloody Diarrhea, cramps, fever, and vomiting	2–10 days	Amperometric	Antigen-antibody	Routine stool culture; Campylobacter requires special media and 42 °C incubation to grow	Diarrhea dysentry
Listeria monocytogenes	Cheeses	Fever, muscle aches, and nausea or diarrhea. Pregnant women may have mild flu-like illness, and infection can lead to premature delivery or stillbirth. Elderly or immunocompromised patients may have bacteremia or meningitis.	2–30days	BIAcore 3000 biosensor (SPR)	Polyclonal antibody	Blood or cerebrospinal fluid Cultures; Asymptomatic fecal carriage occurs; therefore, stool culture usually not helpful	

used for the bacterial immobilization of specific oligonucleotides and for their simultaneous monitoring of hybridization outcomes to form a genuine sensor. Lermo et al. (2007) have demonstrated genomagnetic assay based on *in situ* DNA amplification by use of magnetic primers. Additionally, Elsholz et al. (2006) have devised a PCR free method. In it an electrical 16S rRNA specific oligonucleotide microarray and automated analysis system was devised for *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *S. aureus* and *Staphylococcus epidermidis*. The thiol-modified oligonucleotides with three unlabelled oligonucleotides and biotin labelled oligonucleotides are immobilized on IDA electrodes (interdigitated gold array) for successful fabrication of a sensor. These supporting sequences will lead to improvement in hybridization events. Biotin-labelled oligonucleotides binds with avidin alkaline phophatase enzyme conjugates and the p-aminophenol was thus, liberated by the activity of phosphatase. The electrical signals generated through amperometric redox cycling were detected by multipotentiostat-system. An increase of two more biotins via supporting oligonucleotides will lead to increase in sensitivity of assay by 60%. Additionally, it removes the need of amplification by PCR and thus, is a relatively fast and easy protocol for food borne pathogen detection. Other field-usable RNA-biosensors were also cited based on DNA/RNA hybridization (Baeumner et al., 2003), which are quite capable enough, rapid and sensitive for detection of as low as 40 *E. coli* CFU mL^{-1} from an infected source.

3.10. Potentiometric, FET and LAPS-based biosensors

These are amongst the least common biosensors utilized for pathogen detection. These work on the detection of ions in the solution. For this sake, one inert reference electrode and one working electrode in contact with sample is required. Pathogen detection based on pH changes or ion concentrations fluctuations is possible using these biosensors. These are sensitive generally over a wide range of 10^{-6} to 10^{-1} mol L^{-1} concentration. Another potential advantage is the facility for continuous monitoring provided by them in, *in situ* conditions. These are portable and inexpensive. The major disadvantages associated with these biosensors are the poor selectivity in some environmental samples.

Use of ion-selective field effect transistors (ISFETs) (Bergeveld, 2003) is another approach utilized for biological detection events. These biosensors are fabricated using p-type silicon substrate with two n-doped regions, one acting as a source and other as drain with a gate in between covered by SiO_2 acting as an insulator, which is further, over layered by ion selective membrane. These are newly developed biosensors (Lazcka et al., 2007), but, with reasonably low popularity because of poor detection range and incompatibility of bio molecule immobilization with ISFET fabrication technology.

A new technique for food borne pathogen detection has been evolved from ISFET by combining potentiometry with optical detection. It is known as light-addressable potentiometric sensor (LAPS) (Hafeman et al., 1988). Coupling of a transient photocurrent induced by intensity-modulating light source like light emitting diode (LED) has performed to engage an insulated p/n-doped silicon electrode. Commercially, threshold immunoassay system was utilized for bacterial detection (Dill et al., 1999). Magnitude of induced photocurrent is governed by the potential applied across the silicon plate. However, if different light sources are overlaid on different regions of the electrode, different physiochemical processes can be detected, by using a single core device. Gehring et al. (1998) have developed an immunoligand-based assay (ILA) for detection of *E. coli* O157:H7 cells in buffer. In it, indirect labelling with urease labelled antifluorescein antibody was done and bacterial analyte was sandwiched between biotinylated and fluoresceinated antibodies. Capturing the active immunocomplex, leads to blockage of nitrocellulose filter membrane with streptavidin. Ercole et al. (2003) have demonstrated detection of *E. coli* in vegetable foods. Presence of *E. coli* in food samples is the bioindicator of faecal contamination in food. By using LAPS transducing element in potentiometric alternating biosensing (PAB) system, *E. coli* was measured by detecting variation in pH caused by NH_3 (NH_3 is being produced by urease-*E. coli* antibody conjugate). Liquid phase is achieved by washing the commercial samples of vegetables like lettuce, sliced carrots, and rucola with peptone water. The phase is further separated by blending in sonicator to get bacterial cells detached from food items. Finally, this left over liquid phase has been recorded by PAB system as the fast and sensitive method for the detection of 10 cells mL^{-1} in 1.5 h.

3.11. Impedimetric and conductimetric biosensor

It is a powerful technique used for the detection of electrochemical systems. A small amplitude sinusoidal excitation applied to the system measures the changes in electrical impedance of the medium. Analysis is done on the basis of changes in conductance, capacitance and impedance. Usually due to microbial metabolic processes, conductance and capacitance increases while impedance will decreases (Invitski et al., 2000). Around one century ago, first impedance measurement was carried out for detecting the growth of microorganisms (Stewart, 1899). Detection of *Salmonella* amongst the various food borne pathogens is the one of the major research focus of impedance technology. Association of Analytical Communities International (AOAC) in 1992 (Gibson et al., 1992) has accepted impedance technique as initial screening method for *Salmonella* in food and as a final method of action for *Salmonella* in 1996 (AOAC, 1996). *Enterobacteriaceae*, coliforms, *Listeria* spp., and *L. monocytogenes* can also be detected using impedance microbiological techniques. It is the most successful way out amongst the recently developed automated detection methods and suits well for detecting bacteria in clinical samples to monitor the quality of food. A number of samples can be analyzed at a single time using impedance microbial technology but the sensitivity of impedomertric sensors is less as compared to other sensors. High-density microelectrode array biosensor was fabricated using silicon with 2-μm layer of thermal oxide as an insulating layer with an active area of 9.6 mm^2. It consists of gold fabricated electrode array for the detection of *E. coli* O157:H7 (Radke and Alocilja, 2005). Moreover, heterofunctional cross linkers and immobilized polyclonal antibodies were also used for bacterial detection. Bacteria immobilized on these surfaces, leads to changes in impedance. *E. coli* detection in pure cultures and inoculated samples was performed using this detector system and it was found that this device could easily discriminate between bacterial cell concentrations of 10^4–10^7 CFU mL^{-1}. For detecting *Bacillus cereus* in food, direct charge transfer (DCT) biosensor was developed by Pal et al. (2007). Polyaniline nanowires work as critical transducers and antibodies as potential sensing molecules in the senor system. Detection can be done through the antigen-antibody interactions; and subsequent direct electron flow generates the resistance signals. The assay takes 6 min and the bio sensitivity of pure culture was found out to be 10^1–10^2 CFU mL^{-1}. It is also capable of detecting *B. cereus* from mixed culture having different *Bacillus* species.

4. Conclusion

An early warning system for timely recognition of emerging infections is required to prevent the epidemics. The on time initiated research, awareness, and preventive measures can hinder their effect before they become prevalent public health troubles. This paper reviews some of the most commonly used biosensor systems based on their transducer properties, which include

optical, surface plasmon resonance (SPR), amperometric, potentiometric, whole-cell, electrochemical, impedimetric, piezoelectric and their applications for the rapid detection of pathogens in food. The best amongst all is multi-array biosensor system which detects multiple pathogens in very short period of time. The suitable biorecognition element (enzymes, antibodies or nucleic acids) is the better judge for the type of biosensor configuration used in food borne pathogen detection. It also highlights that the best indicator of the success of a biosensor will rely on the success of emerging sophisticated micro and nanotechnologies as well as biology, biochemistry, chemistry, physics, and electronics. To accomplish this goal, important investment in research infrastructure, subject expertise, and the essential lab-facilities are required (Collings and Caruso, 1997). However, as the world becomes more concerned about the impact of food on public health, the safety against biowar and the demand for rapid detection of biosensors has elevated commercially. The conventional traditional methods are time-consuming and tedious for the detection and identification of microbial contaminants from food. So, an exciting modern era alternative research has focused on the development of biological sensors for the detection of pathogens. Biosensors offer rapid, real time and multiple analyses from perishable or semi-perishable foods. However, applying biosensors also has some limitations in its sensitivity, cost and the need for sample pre-treatment for the detection of pathogens. The combined research efforts are in progress globally after the release report of CDC (Centers for Disease and Prevention/Control) on nationally notifiable infectious diseases by food. The CDC United States 2007, 2009 report, has recognized anthrax (gastrointestinal), botulism (foodborne), cholera, coccidioidomycosis, cryptosporidiosis, cyclosporiasis, giardiasis, hemolytic uremic syndrome, post-diarrheal, hepatitis-A, acute listeriosis, salmonellosis, shiga toxin-producing *E. coli* (STEC) shigellosis, trichinellosis (trichinosis), and typhoid fever as the most potential food borne life-threats. Future researches on developing biosensors for these diseases may help to fight from the food borne epidemics at a glance.

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