



Research review paper

An overview of foodborne pathogen detection: In the perspective of biosensors

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ARTICLE INFO

Article history:

Received 9 July 2008

Received in revised form 3 December 2009

Accepted 4 December 2009

Available online 16 December 2009

Keywords:

Foodborne pathogen

Conventional methods

Biosensors

Bioreceptors

Optical transducer

Electrochemical transducer

ABSTRACT

Food safety is a global health goal and the foodborne diseases take a major crisis on health. Therefore, detection of microbial pathogens in food is the solution to the prevention and recognition of problems related to health and safety. For this reason, a comprehensive literature survey has been carried out aiming to give an overview in the field of foodborne pathogen detection. Conventional and standard bacterial detection methods such as culture and colony counting methods, immunology-based methods and polymerase chain reaction based methods, may take up to several hours or even a few days to yield an answer. Obviously this is inadequate, and recently many researchers are focusing towards the progress of rapid methods. Although new technologies like biosensors show potential approaches, further research and development is essential before biosensors become a real and reliable choice. New bio-molecular techniques for food pathogen detection are being developed to improve the biosensor characteristics such as sensitivity and selectivity, also which is rapid, reliable, effective and suitable for *in situ* analysis. This paper not only offers an overview in the area of microbial pathogen detection but it also describes the conventional methods, analytical techniques and recent developments in food pathogen detection, identification and quantification, with an emphasis on biosensors.

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1. Introduction

1.1. Food and health legislations

The food industry is the main party concerned with the presence of pathogenic microorganisms, where failure to detect a pathogen may lead to a dreadful effect. Although the safety of food has dramatically improved overall, progress is uneven and foodborne outbreaks from microbial contamination, chemicals and toxins are common in many countries (WHO, 2007b). International trade statistics (2007) by World Trade Organization (WTO) reported that Europe has accounted for 46% of world exports of agricultural products, where food represents 80% of agricultural exports (WTO, 2007). Trading of contaminated food between countries increases the potential for outbreaks and consequently, health risks posed by microbial pathogens in food are of major concern to all governments.

In November 2007, U.S. Food and Drug Administration (FDA) has developed a comprehensive 'Food Protection Plan', in which it has been mentioned that food must be considered as a potential vehicle for intentional contamination (FDA, Food Protection Plan, 2007). Such intentional contamination of food could result in human or animal illnesses and deaths, as well as economic losses.

It has been reported in the EU legislation on microbiological criteria for foodstuffs, that "foodstuffs should not contain microorganisms or their toxins or metabolites in quantities that present an unacceptable risk for human health", as laid down in Regulation (EC) No 2073/2005 (Regulation (EC), 2005). Recently, the World Health Assembly (WHA) established a global surveillance system for public health emergencies of international concern by adopting the International Health Regulations (IHR) on 23 May 2005 which came into force on 15 June 2007 (WHO, 2005).

All these current legislations on food and health provide an intense inspiration into the area of food pathogen detection. Therefore, a comprehensive literature survey has been carried out aiming to give an overview of the field of foodborne pathogen detection. First, some of the outbreaks caused by foodborne pathogens and the main pathogens that cause foodborne diseases are discussed. Next, the main conventional methods in pathogen detection are described, covering their strengths and weaknesses. Then the role of biosensors in the field of foodborne pathogen detection is analysed, comprising all main types. Since, the literature related to foodborne pathogens is vast; this paper reports on recent advances mainly on the detection, identification and quantification of pathogens, with an emphasis on biosensors.

1.2. Emerging foodborne pathogens

The World Health Organization (WHO) defines foodborne illnesses as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food. Though the global incidence of foodborne disease is difficult to estimate, it has

been reported that in 2005 alone 1.8 million people died from diarrhoeal diseases and a great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2007a). In industrialized countries, the percentage of the population suffering from foodborne diseases each year has been reported to be up to 30%. For, example, the Centers for Disease Control and Prevention (CDC, 2005) estimated that around 76 million cases of foodborne diseases, resulting in 325,000 hospitalizations and 5000 deaths occur each year in the USA.

Some foodborne diseases are well recognized, but are considered emerging because they have recently become more common. Though there are various food borne pathogens that have been identified for food borne illness, *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 have been generally found to be responsible for majority of food-borne outbreaks (Alocilja and Radke, 2003; Chemburu et al., 2005). For example, in Ireland, *Campylobacter* is the most important cause of sporadic cases of foodborne illness, with 1815 cases of *Campylobacter* infection reported in 2006 (42.8/100,000 population), which was over four times the number of Salmonellosis cases reported in the same year (FSAI, 2006). Also, most of the earlier and recent food products recalls are also due to these pathogens (Belson and Fahim, 2007). List of pathogenic microorganisms responsible for foodborne illness and outbreaks caused by them are given in Tables 1 and 2 respectively.

There are many methodical programs like good agricultural practices (Kay et al., 2008; Umali-Deininger and Sur, 2007), good manufacturing practices (Mucchetti et al., 2008; Umali-Deininger and Sur, 2007), hazard analysis and critical control point (HACCP) (Jin et al., 2008; Taylor, 2007) and the food code indicating approaches (Piatek and Ramaen, 2001), which can significantly reduce the pathogenic microorganisms in food. But still, the role of pathogen detection technology is vital, which is the key to the prevention and identification of problems related to health and safety. Next, the traditional methods employed for foodborne pathogen detection over the past decades to the recent year will be discussed, by highlighting their strengths and weakness.

2. Various methods towards pathogen detection

Conventional methods for the detection and identification of microbial pathogenic agents mainly rely on specific microbiological and biochemical identification. Conventional methods being used for the detection of pathogens are illustrated in Fig. 1, where the culture and colony counting methods involve counting of bacteria, immunology-based methods involve antigen–antibody interactions and the third polymerase chain reaction (PCR) method which involves DNA analysis. While these methods can be sensitive, inexpensive and give both qualitative and quantitative information of the tested microorganisms, they are greatly restricted by assay time, also initial enrichment is needed in order to detect pathogens which typically occur in low numbers in food.

Table 1
Pathogenic microorganisms responsible for foodborne illness.

Microorganism	Associated foods (reported food contaminants)	Infective dose ^a V (no. of organisms)	Incubation period ^b	Symptoms	Name of the disease	References
<i>Campylobacter jejuni</i>	Raw milk, and raw or under-cooked meat, poultry or shellfish.	400–500	2 to 5 days	Fever, headache, and muscle pain followed by diarrhea, abdominal pain and nausea	Campylobacteriosis	(Bosilevac et al., 2007; Chai et al., 2007; Cole et al., 2006; Dao and Yen, 2006; Esteban et al., 2008; Freitas and Noronha, 2007; Gallay et al., 2008; Gellynck et al., 2008; Ghafir et al., 2007; Gormley et al., 2008; Hussain et al., 2007; Jayarao et al., 2006; Kegode et al., 2008; Kiess et al., 2007; Little et al., 2008c; Melly et al., 2007; Mena et al., 2008; Oermeci and Oezdemir, 2007; Sallam, 2007; Stoyanchev et al., 2007; Sulonen et al., 2007; Unicomb et al., 2008; Vindigni et al., 2007; Whyte et al., 2005; Wong et al., 2007; Workman et al., 2008; Yoda and Uchimura, 2006)
<i>Salmonella</i> spp.	Raw/undercooked eggs, poultry, and meat; raw milk and dairy products; seafood; chocolate; salad and spices	15–20	12 to 24 h	Stomach pain, diarrhea, nausea, chills, fever, and headache	Salmonellosis	(Abadias et al., 2008; Atanassova et al., 2008; Bosilevac et al., 2007; Brandl and Amundson, 2008; Cabedo et al., 2008; Chan and Chan, 2008; Christison et al., 2008; Dao and Yen, 2006; Eglezos et al., 2008; Esteban et al., 2008; Esteves et al., 2008; Gadaga et al., 2008; Kegode et al., 2008; Khaita et al., 2007; Knutson et al., 2006; Little et al., 2008c; Mitzscherling and Kuhne, 2008; Pinu et al., 2007; Su et al., 2005a; Urabe et al., 2008; Van et al., 2007; Vasavada, 2004; Vindigni et al., 2007; Vojdani et al., 2008)
<i>E. coli</i>	Raw/undercooked eggs, poultry, and meat; raw milk and dairy products; seafood; and leafy vegetables	< 10	2 to 4 days	Stomach pain, diarrhea, nausea, chills, fever, and headache	Hemorrhagic colitis	(Abadias et al., 2008; Alexandre and Prado, 2003; Atanassova et al., 2008; Brandl and Amundson, 2008; Christison et al., 2008; Dao and Yen, 2006; Eglezos et al., 2008; Jayarao et al., 2006; Kegode et al., 2008; Kim and Harrison, 2008; Knutson et al., 2006; Little et al., 2008b; Macdonald et al., 1985; Manges et al., 2007; Manning et al., 2008; Najand and Ghanbarpour, 2006; O'Beirne, 2007; Ramaswamy et al., 2008; Sehgal et al., 2008; Valentin-Bon et al., 2008; Vasavada, 2004; Vojdani et al., 2008; Warren et al., 2008)
<i>L. monocytogenes</i>	Soft cheese, raw milk, improperly processed ice cream, raw leafy vegetables; raw meat and poultry	< 1000	2 days to 3 weeks	Fever, chills, headache, backache, sometimes abdominal pain and diarrhea	Listeriosis	(Abadias et al., 2008; Atanassova et al., 2008; Bosilevac et al., 2007; Cabedo et al., 2008; Christison et al., 2008; Esteban et al., 2008; Gounadaki et al., 2008; Jayarao et al., 2006; Kvenberg, 1988; Little et al., 2008a; Little et al., 2008b; Neves et al., 2008; Sireli and Gucukoglu, 2008; Sumner and Ross, 2002; Vasavada, 2004)
<i>Bacillus cereus</i>	Meats, milk, vegetables, fish, rice, pasta, and cheese	> 10 ⁶ /g	30 min to 15 h	Diarrhea, abdominal cramps, nausea, and vomiting	<i>Bacillus cereus</i> food poisoning	(Christison et al., 2008; Fricker et al., 2008; Kim et al., 2004; King et al., 2007; Kislá and Uzgun, 2008; Kivaria et al., 2006; Lapidus et al., 2008; Lee et al., 2007; Portnoy et al., 1976; Warminska-Radyko et al., 2007; Yokoyama et al., 1999)

Table 1 (continued)

Microorganism	Associated foods (reported food contaminants)	Infective dose ^a V (no. of organisms)	Incubation period ^b	Symptoms	Name of the disease	References
<i>Clostridium botulinum</i>	Improperly canned foods, garlic in oil, and vacuum-packaged and tightly wrapped food	< nano grams	12–36 h	Double vision, droopy eyelids, trouble speaking and swallowing, and difficulty breathing	Foodborne botulism	(Arritt et al., 2007; Bianco et al., 2008; Carlin et al., 2004; Cereser et al., 2008; Dressler, 2005; Gdynia et al., 2007; Kimura et al., 2008; Kissani et al., 2007; Kuplulu et al., 2006; O'Beirne, 2007; Peck, 2006; Ragazani et al., 2008; Shuler et al., 2006; Sumner and Ross, 2002; Wiwanitkit, 2006)
<i>Clostridium perfringens</i>	Undercooked meats, meat products, and gravies	>10 ⁸	8–22 h	Abdominal cramps and diarrhea	Perfringens food poisoning	(Esteves et al., 2008; Juneja et al., 2006; Lee et al., 2007; Maslanka et al., 1999)
<i>Shigella</i>	Salads, raw vegetables, dairy products, and poultry	<10	12–50 h	Abdominal pain, cramps, fever, vomiting, and diarrhea containing blood and mucus	Shigellosis	(Agle et al., 2005; Chanachai et al., 2008; Ghosh et al., 2007; Kimura et al., 2006; Pinu et al., 2007; Selma et al., 2007; Warren et al., 2007)
<i>Yersinia enterocolitica</i>	Meat (mostly pork), oysters, fish, and raw milk	Unknown	1–3 days	Diarrhea and/or vomiting; fever and abdominal pain	Yersiniosis	(Arnold et al., 2006; Bhaduri et al., 2005; Fredriksson-Ahomaa et al., 2006; Fredriksson-Ahomaa et al., 2007; Gonc et al., 2007; Grahek-Ogden et al., 2007; Jayarao et al., 2006; Kasalica and Miocinovic, 2004; Selma et al., 2006; Siriken, 2004; Vasavada, 2004; Yucel and Ulusoy, 2006)
<i>Vibrio parahaemolyticus</i>	Raw, improperly cooked, or cooked, recontaminated fish and shellfish, and oysters	> 1 million	4 h–4 days	Diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills	<i>V. parahaemolyticus</i> associated gastroenteritis	(Chan and Chan, 2008; Cohen et al., 2007; Davis et al., 2007; Gopal et al., 2005; Kudaka et al., 2008; Lhafi and Kuhne, 2007; Mahmud et al., 2007; Mitzscherling and Kuhne, 2008; Normanno et al., 2006; Su et al., 2005a; Sumner and Ross, 2002; Zimmerman et al., 2007)
<i>Vibrio vulnificus</i>	Raw or recontaminated oysters, clams, and crabs	<100	<16 h	Diarrhea, and wound infections	Syndrome called “primary septicemia”	(Colakoglu et al., 2006; Gopal et al., 2005; Jung et al., 2007; Lhafi and Kuhne, 2007; Mitzscherling and Kuhne, 2008; Normanno et al., 2006; Sumner and Ross, 2002; Wong and Liu, 2008)
<i>Hepatitis A virus</i>	Sandwiches, fruits and fruit juices, milk and milk products, vegetables, salads, shellfish, and iced drinks	10–100	Unknown	Fever, malaise, nausea, anorexia, and abdominal discomfort, followed by jaundice	Hepatitis A	(Bialek et al., 2007; Croci et al., 2005; Frank et al., 2007b; Greening, 2006; Hasegawa et al., 2007; Macaluso et al., 2006; Sanchez et al., 2007; Shieh et al., 2007; Sincero et al., 2006; Wheeler et al., 2005)
<i>Norwalk virus/Norovirus</i>	Raw oysters/shellfish, water and ice, salads, and frosting	Unknown but presumed to be low	1–2 days	Nausea, vomiting, diarrhea and abdominal cramps	Viral gastroenteritis, “stomach flu” or “winter vomiting disease”	(David et al., 2007; De Wit et al., 2007; Greening, 2006; Le Guyader et al., 2006; Maekawa et al., 2007; Mitzscherling and Kuhne, 2008; Nenonen et al., 2008; Thongsawad et al., 2007; Webby et al., 2007)

^a Infective dose: the amount of agent that must be consumed to give rise to symptoms of foodborne illness.

^b Incubation period: the delay between consumption of a contaminated food and appearance of the first symptoms of illness.

Table 2

Recent foodborne outbreaks caused by pathogenic microorganisms.

Pathogen	No of illness	Consumed food	Place and year of outbreak	Source of the outbreak	Reference
<i>Campylobacter jejuni</i>	33	Cooked chicken	High school in Japan, 2005	Secondary contamination in cooking practice	(Yoda and Uchimura, 2006)
<i>Salmonella</i> spp.	72	Egg, squash and seafood	Funeral service in South Korea, 2007	Infected food handler and/or the contaminated food	(Kim et al., 2007b)
<i>Salmonella</i> spp.	111	Cake	Nursing home in Germany, 2006	High ambient summer temperatures and failure to keep the cake refrigerated	(Frank et al., 2007a)
<i>Salmonella</i> spp.	46	Confectionary (éclairs)	Sports event in Berlin, Germany, 2006	Insufficient cooling of the éclairs during transport and before sale could have enhanced bacterial growth	(Wichmann-Schauer et al., 2006)
<i>Salmonella</i> spp. and <i>Shigella</i>	103	Mixed rice and chicken dish	School in Bangkok, and Thailand, 2005	Mixed contamination	(Chanachai et al., 2008)
<i>Salmonella</i> spp.	56	Pre-processed raw beef	Netherlands, 2005	Imported contaminated beef	(Kivi et al., 2007)
<i>Salmonella</i> spp.	21	Rucola lettuce	Norway, 2004	Imported rucola lettuce	(Nygard et al., 2008)
<i>Salmonella</i> spp.	98	Chicken with harusame (bean-jelly)	A nursery school in Kitakyushu City, Japan, 2004	Infected raw eggs contaminated the food through the food handlers hands and cooking utensils	(Tokuzaki and Takahashi, 2007)
<i>Salmonella</i> spp.	125	Cheese, lettuce and roma tomatoes	Restaurant in north-eastern United States, 2004	Contaminated roma tomatoes	(Gupta et al., 2007)
<i>Salmonella</i> spp.	1435 cases and 117 hospitalizations	Hard pastry with vanilla cream	During a festival in Catalonia, Spain, 2002	Inadequate handling of foods containing eggs	(Camps et al., 2005)
<i>E. coli</i> O157:H7	30	Hamburger patties	USA, 2007	Contaminated beef patties distributed by the meat processing plant	(Belson and Fahim, 2007)
<i>E. coli</i> O103 : H25	17cases and one death	Cured mutton sausage	Norway, 2006	Contaminated mutton	(Schimmer et al., 2008)
<i>L. monocytogenes</i>	54 cases; 8 deaths, and 3 fetal deaths	Turkey deli meat	USA, 2002	Contaminated turkey distributed by turkey processing plant	(Gottlieb et al., 2006)
<i>L. monocytogenes</i>	86	Cheese	Japan, 2001	Contaminated cheese products distributed by the cheese producing plant	(Makino et al., 2005)
Norovirus	26 confirmed and 53 clinical cases	Raw oysters	British Columbia, Canada, 2004	Not specified	(David et al., 2007)
Hepatitis A	601cases and 3 deaths	Green onions	Restaurant in Pennsylvania, USA, 2003	Contaminated green onions distributed to the restaurant	(Wheeler et al., 2005)
Norwalk-like viruses	130	Salad sandwiches	Farewell party in the nurses' hostel of a civil hospital in Delhi, India, 2002	Not specified	(Girish et al., 2002)

2.1. Culture and colony based methods

Conventional culture methods remain the most reliable and accurate techniques for food-borne pathogen detection. Although the culture based methods are found to be standard microbiological techniques to detect the single bacteria, amplification of the signal is required through growth of a single cell into a colony. Culture methods have been used for detection of *L. monocytogenes* (Artault et al., 2001; DeBoer and Beumer, 1999; Stephan et al., 2003), *S. aureus*, *Salmonella*, *Coliforms*, *E. coli*, etc. (Ayçiçek et al., 2004), *Campylobacter jejuni* (Sanders et al., 2007) and *Yersinia enterocolitica* (Weagant, Feb 2008) etc. Some standard methods for the detection of *L. monocytogenes* can require up to 7 days to yield results, as they rely on the ability of microorganisms to multiply to visible colonies (Artault et al., 2001; DeBoer and Beumer, 1999). In addition, viable bacterial strains in the environment can enter a dormancy state where they become non-

culturable (viable-but non-culturable (VBNC)) which can subsequently lead to an underestimation of pathogen numbers or a failure to isolate a pathogen from a contaminated sample (Porter et al., 1995; Toze, 1999; Xu et al., 1982). Detection of *L. monocytogenes* performed in a two-step cultural enrichment process took on average one week until the biochemical identification of the *L. monocytogenes* suspicious colony is completed (Stephan et al., 2003). The ALOA® method (AES Laboratoire), which uses a chromogenic medium in conjunction with a *Listeria* monodisk for the detection of *L. monocytogenes*, can reduce detection time down to 3 days (Artault et al., 2001). It has been reported that to detect *Campylobacter*, 4–9 days are needed to obtain a negative result and between 14 and 16 days for confirmation of a positive result (Brooks et al., 2004).

The major drawbacks of microbiological methods are their labour-intensiveness and are time-consuming as it takes 2–3 days for initial results, and up to 7–10 days for confirmation. This is an obvious inconvenience in many industrial applications, particularly in the field of foods sector. In spite of their disadvantages, conventional culture methods still represent a field where progress is possible. These methods are often combined together with other pathogen detection methods like an automated or semi-automated DNA, antibody, or biochemical-based method to yield more robust results. Examples include the Vermicon identification technology (VIT®) a rapid commercial test system compared to a cultural standard method for *Listeria* (Stephan et al., 2003); Comparison of ELISA and PCR vis-a-vis cultural methods for detecting *Aeromonas* spp. (Arora et al., 2006). The detection of *Salmonella* spp. (Lofstrom et al., 2004; Murphy et al.,

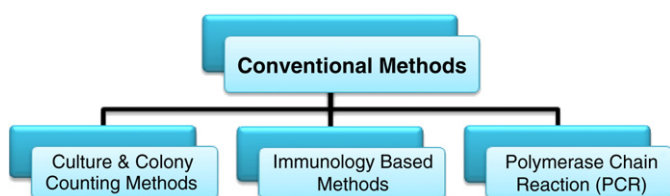


Fig. 1. Different conventional methods employed for pathogen detection.

2007), *L. monocytogenes* (Murphy et al., 2007) and *Campylobacters* (Josefsen et al., 2004; Magistrado et al., 2001) by PCR after culture enrichment has been reported. Also, culture and BAX PCR-based detection for *Listeria* spp. (Shearer et al., 2001) and *L. monocytogenes* (Hoffman and Wiedmann, 2001; Shearer et al., 2001) *Salmonella enteritidis* (Shearer et al., 2001), *E. coli* O157: H7 (Shearer et al., 2001) have been reported.

2.2. Immunology-based methods

Immunological detection with antibodies is perhaps the only technology that has been successfully employed for the detection of bacterial cells, spores, viruses and toxins alike (Iqbal et al., 2000). Methods based on antigen–antibody bindings are widely used for determining food-borne pathogens. Immunology-based methods has been used for the detection of *Escherichia coli* (Abdel-Hamid et al., 1999b; Aldus et al., 2003; Chen and Durst, 2006; Gehring et al., 2006; Magliulo et al., 2007; Sunwoo et al., 2006), *Salmonella* (Abdel-Hamid et al., 1999b; Chen and Durst, 2006; Magliulo et al., 2007; Schneid et al., 2006; Valdivieso-Garcia et al., 2003), *L. monocytogenes* (Chen and Durst, 2006; Churchill et al., 2006; Gangar et al., 2000; Hibi et al., 2006; Hudson et al., 2001; Jung et al., 2003; Magliulo et al., 2007; Sewell et al., 2003; Yu et al., 2004b), *Staphylococcal enterotoxins* (Bennett, 2005; Jechorek and Johnson, 2008; Rasooly and Rasooly, 1998; Schlosser et al., 2007), *Campylobacter* spp. (Borck et al., 2002; Che et al., 2001; Hochel et al., 2007; Siragusa et al., 2001). In order to ensure the reliable detection of foodborne pathogens using antibody-based methods, the influence of stress on antibody reactions should be thoroughly examined and understood first as the physiological activities in cells are often altered in response to a stress (Hahm and Bhunia, 2006).

The number of antibody types and formats are available for immunodetection. These include conventional and heavy chain antibodies, as well as polyclonal, monoclonal or recombinant antibodies. Polyclonal antibodies can be raised quickly and cost effectively associated with the production of monoclonal antibodies (Cahill et al., 1995) and it has been used as detection vehicles for several decades (Breitling and Dübel, 1999). Polyclonal antibodies are able to detect *L. monocytogenes* (Feldsine et al., 1997a; Jung et al., 2003), *Campylobacter* species (Hochel et al., 2007), *Fusarium* sp. – a primary corn pathogen (Meirelles et al., 2006). However, polyclonal antibodies are limited both in terms of their specificity and abundance (Leonard et al., 2003). Since the progress of hybridoma techniques (Kohler and Milstein, 1975) and the emergence of recombinant antibody phage display technology, developed during the past decade, immunological detection of microbial contamination has become more sensitive, specific, reproducible and reliable with many commercial immunoassays available for the detection of a wide variety of microbes and their products (Leonard et al., 2003).

Monoclonal antibodies are often more useful for specific detection of a molecule than polyclonal antibodies because they provide an indefinite supply of single antibody. Monoclonal antibodies are used to detect wide range of pathogens in food. Examples are *L. monocytogenes* [14, 32–36], and *Listeria innocua* (Solve et al., 2000), *Escherichia coli* O157 (Zhao and Liu, 2005), *Salmonella enterica* (Schneid et al., 2005), *Salmonella typhi* (Kumar et al., 2003), *Staphylococcus aureus* enterotoxins (Deckenback et al., 2002), *Shigella* (Warren et al., 2006), zearalenone – a worldwide occurring pathogen in cereal grains (Kawamura and Emoto, 2006) etc. An immunochromatography (ICG) strip test based on a monoclonal antibody for the detection of *L. monocytogenes* showed results within 20 min without complicated steps (Shim et al., 2007). In spite of their number of advantages over polyclonal antibodies, the monoclonal antibodies have few disadvantages as well. The problems with monoclonal antibodies are, there is a need for a skilled technician and specialized growth apparatus for tissue culturing, and also expensive to produce. But recombinant anti-

bodies fill this void since they can be produced in reasonable quantities in short periods of time from bacterial expression systems.

While the immunological-based detection is not much specific and sensitive than nucleic acid-based detection, but it is faster, more robust and has the ability to detect not only contaminating organisms but also their biotoxins that may not be expressed in the organism's genome (Iqbal et al., 2000). Examples of immunological techniques include the enzyme immunoassay (EIA) (Borck et al., 2002), enzyme-linked immunosorbent assay (ELISA) (Bennett, 2005; Beumer and Brinkman, 1989; Mattingly et al., 1988; Palumbo et al., 2003), flow injection immuno-assay (Abdel-Hamid et al., 1999b), enzyme-linked fluorescent assay (ELFA) [11, 13], bioluminescent enzyme immuno-assay (BEIA) (Valdivieso-Garcia et al., 2003), enzyme-linked immunomagnetic chemiluminescence (ELIMCL) (Gehring et al., 2006) immunochromatography (ICG) strip test (Shim et al., 2007), immunomagnetic separation (Dickson and Chen, 2001; Hudson et al., 2001; Refseth et al., 2001), immuno-precipitation assay (Feldsine et al., 1997b), agglutination test (Matar et al., 1997), radio-immunoassays (RIA), western blot test (Rasooly and Rasooly, 1998) and technically modified western blot include the line immunoassay (LIA) and the recombinant immunoblot assay (RIBA).

In spite of their very less assay time compared to traditional culture techniques, antibody-based detection is still lacking the ability to detect microorganisms in “real-time”. In addition, if quantities of the pathogen are high enough then immunoassays can be used to provide real-time information. But the problems that may arise with immunoassay-based methods are the low sensitivity of the assays, low affinity of the antibody to the pathogen or other analyte being measured, and potential interference from contaminants (Meng and Doyle, 2002). Immunology-based methods found coupled with other methods for pathogen detection, like immunomagnetic separation on magnetic beads is coupled with matrix-assisted laser desorption ionization-time of flight mass spectrometry for detection of staphylococcal enterotoxin B (Schlosser et al., 2007), combination of immunomagnetic separation with flow cytometry for detection of *L. monocytogenes* (Hibi et al., 2006; Jung et al., 2003).

2.3. Polymerase chain reaction (PCR)

Techniques to detect a single bacterium were realized almost 20 years ago with the invention of the polymerase chain reaction (PCR). This method can detect a single copy of a target DNA sequence, and thus, can be used to detect a single pathogenic bacterium in food. It is promising because it detects the organism by amplifying the target rather than the signal, and is therefore less prone to producing false-positives. A target DNA can be amplified 1-million-fold in less than an hour, with sensitivities in theory down to a single target pathogen (A.Batt, 2007). So PCR is a very widely used detection method for the pathogens in food, forming the basis for detection systems utilizing nucleic acid. Also it can be used to enhance the sensitivity of nucleic acid-based assays.

PCR has distinct advantages over culture and other standard methods for the detection of microbial pathogens and offers the advantages of specificity, sensitivity, rapidity, accuracy and capacity to detect small amounts of target nucleic acid in a sample (Toze, 1999). Recently it was reported that PCR assay was found to be the most sensitive in the detection of *Salmonella* in seafood when compared to culture, ELISA methods (Kumar et al., 2008). Lower detection rate with culture and ELISA assays could be attributed to greater sensitivity of the PCR method. Also when testing food samples, false negative PCR results can occur and may be due to interference with target-cell lysis necessary for nucleic acid extraction, nucleic acid degradation and/or direct inhibition of the PCR. Therefore, it is essential to include appropriate controls for the application of PCR to the detection of pathogens in food samples (Murphy et al., 2007).

PCR based methods are used in the detection wide range of pathogens like *S. aureus* (Kim et al., 2007a; Riyaz-UI-Hassan et al., 2008), *L. monocytogenes* (Chen and Knabel, 2007; Kim et al., 2007a; Murphy et al., 2007; O'Grady et al., 2008; Oravcova et al., 2007; Perry et al., 2007), *Salmonella* (Choi and Lee, 2004; Kim et al., 2007a; Malorny et al., 2007; Murphy et al., 2007; Perry et al., 2007; Stark and Made, 2007), *Bacillus cereus* (Messelhauser et al., 2007; Perry et al., 2007), *Escherichia coli* O157: H7 [54, 58], *Yersinia enterocolitica* (Perry et al., 2007), *C. jejuni* (Ronner and Lindmark, 2007). The different PCR based methods used to detect pathogens are real-time PCR (Desai et al., 2008; Malorny et al., 2008; O'Grady et al., 2008; Ronner and Lindmark, 2007), multiplex PCR [54, 57, 66] and reverse transcriptase PCR (RT-PCR) (Choi and Lee, 2004). RT-PCR also is found as multiplex RT-PCR (Loisy et al., 2005; Nordstrom et al., 2007; Wolf et al., 2007) and real-time RT-PCR (Casas et al., 2007; Dubois et al., 2007; Loisy et al., 2005).

Compared to other PCR based methods, multiplex PCR is very useful as it allows the simultaneous detection of several by introducing different primers to amplify DNA regions coding for specific genes of each bacterial strain targeted (Touren et al., 2005). Examples of multiplex PCR technique for the simultaneous detection pathogens include multiplex PCR assay for rapid and simultaneous detection of *E. coli* O157:H7, *Salmonella*, *S. aureus*, *L. monocytogenes*, and *Vibrio parahaemolyticus* (Kim et al., 2007a), simultaneous detection of bacteria of the genus *Listeria*, *L. monocytogenes*, and major serotypes and epidemic clones of *L. monocytogenes* (Chen and Knabel, 2007) and the simultaneous detection of *E. coli* O157: H7 and *L. monocytogenes* (Mukhopadhyay and Mukhopadhyay, 2007). In recent years real-time PCR based technologies have emerged as a leading technology for rapid identification of pathogens due to their speed and high degree of sensitivity and specificity, also it permits to obtain quicker results without too much manipulation. But one of the main limitations of the above mentioned PCR techniques is that, the users cannot distinguish between viable and non-viable cells, since DNA is always present whether the cell is dead or alive. But this limitation can be overcome by using Reverse Transcriptase PCR (RT-PCR).

Yaron and Matthews (2002) developed an RT-PCR to detect only viable cells of *E. coli* O157:H7. Also they suggested that, if RT-PCR is to be used for detection of live cells in a sample without enrichment, then 10^7 colony-forming units (CFU) of the target organism are required. It was stated (Casas et al., 2007), that real-time RT-PCR yielded higher detection sensitivity than that obtained by conventional RT-nested PCR. Real-time RT-PCR detection eliminates some purification and concentration steps that are required for conventional RT-nested PCR detection. Other than RT-PCR, application of bacteriophages is found to be an alternative technique to detect viable cells (Hagens and Loessner, 2007; Loessner, 2005). The phages or phage-derived proteins not only be used to detect the presence of unwanted pathogens in food or the production environments but also allows quick and specific identification of viable cells.

PCR is also being found coupled to other techniques. Examples are a combination of immunofluorescent microscopy and a nested PCR (Moore et al., 2007), PCR – enzyme-linked immunosorbent assay (Gillespie et al., 2003), a combination of immunomagnetic separation with the reverse transcription multiplex TaqMan PCR system (Tsai et al.,

2006) etc. In spite of its advantages, from an industrial point of view routine detection of microbes using PCR can be expensive and complicated, requiring skilled workers to carry out the tests. Other reported analytical methods of pathogen detection include, application of bacteriophages for detection and control of foodborne pathogens (Hagens and Loessner, 2007; Loessner, 2005), DNA oligonucleotide arrays (Quinones et al., 2007), in which specific identification of pathogens was achieved without the need for a PCR amplification.

Since all the traditional methods to detect foodborne pathogens are often time-consuming, there is a need for new technology that is rapid, reliable, simple, specific and sensitive. In addition, it should be able for *in situ* real-time monitoring at a low cost. In recent years, there has been much research activity in the area of biosensor development for detecting pathogenic microorganisms.

Next, an overview of recent efforts using biosensors in the field of food pathogen detection will be given. This overview aims to give a broad picture of the different existing technologies, effective methodologies and recent developments of biosensors in microbial pathogen detection.

3. Biosensors in foodborne pathogen detection

3.1. Introduction to biosensors

A biosensor is an analytical device, which converts a biological response into an electrical signal. It consists of two main components: a bioreceptor or biorecognition element, which recognizes the target analyte and a transducer, for converting the recognition event into a measurable electrical signal. A bioreceptor can be a tissue, microorganism, organelle, cell, enzyme, antibody, nucleic acid and biomimic etc. and the transduction may be optical, electrochemical, thermometric, piezoelectric, magnetic and micromechanical or combinations of one or more of the above techniques.

Fig. 2 shows schematic diagram of a biosensor. The bioreceptor recognizes the target analyte and the corresponding biological responses are then converted into equivalent electrical signals by the transducer. The amplifier in the biosensor responds to the small input signal from the transducer and delivers a large output signal that contains the essential waveform features of an input signal. The amplified signal is then processed by the signal processor where it can later be stored, displayed and analysed. Biosensors have been widely applied to a variety of analytical problems in medicine, the environment, food, process industries, security and defence.

3.2. Classifications of biosensor

Biosensors can be classified by their bioreceptor or their transducer type. Fig. 3 shows the biosensor classifications.

4. Bioreceptors

Bioreceptors or the biological recognition elements are the key to specificity for biosensor technologies. A bioreceptor is a molecular species that utilizes a biochemical mechanism for recognition. They

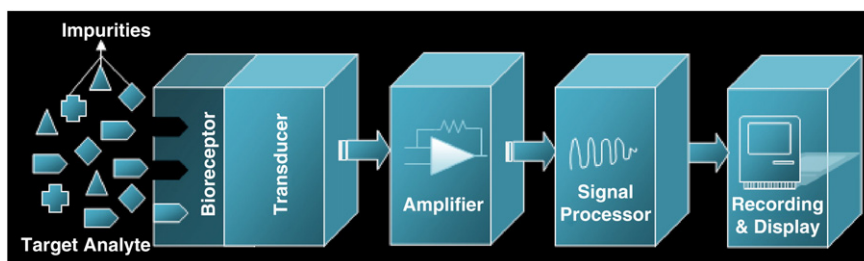


Fig. 2. Schematic diagram of a biosensor.

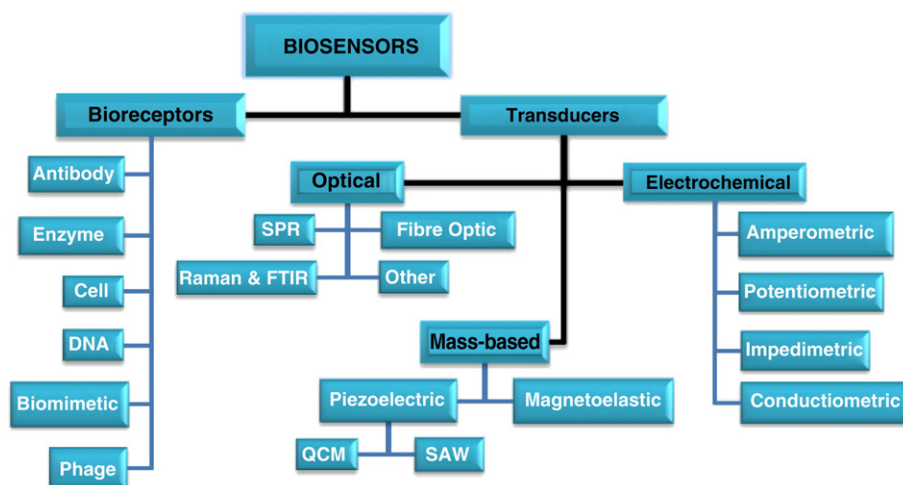


Fig. 3. Classifications of biosensor.

are responsible for binding the analyte of interest to the sensor for the measurement.

Bioreceptors can generally be classified into five different major categories. These categories include antibody/antigen, enzymes, nucleic acids/DNA, cellular structures/cells, biomimetic and bacteriophage (phage). The enzymes, antibodies and nucleic acids are the main classes of bioreceptors which are widely used in biosensor applications. Though the enzymes are one of the biorecognition elements, they are mostly used to function as labels than the actual bioreceptor.

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4.1. Antibody bioreceptors

Antibodies are common bioreceptors used in biosensors. Antibodies may be polyclonal, monoclonal or recombinant, depending on their selective properties and the way they are synthesized. In any case, they are generally immobilized on a substrate, which can be the detector surface, its vicinity, or a carrier (Lazcka et al., 2007).

The way in which an antigen and an antigen-specific antibody interact is similar to a lock and key fit (Vo-Dinh and Cullum, 2000). An antigen-specific antibody fits its unique antigen in a highly specific manner, so that the three-dimensional structures of antigen and antibody molecules are matching. Due to this three-dimensional shape fitting, and the diversity inherent in individual antibody make-up, it is possible to find an antibody that can recognize and bind to any one of a large variety of molecular shapes. This unique property of antibodies is the key that makes the immunosensors a powerful analytical tool and their ability to recognize molecular structures allows one to develop antibodies that bind specifically to chemicals, biomolecules, microorganisms, etc. One can then use such antibodies as specific probes to recognize and bind to an analyte of interest that is present, even in extremely small amounts, within a large number of other chemical substances. The basic structure of an antibody and antigen–antibody lock and key fit is illustrated in Fig. 4.

Antibodies can be covalently modified in many ways to suit the purpose of a particular assay. Many immunological methods involve the use of labeled antibodies. Enzymes, biotin, fluorophores and radioactive isotopes are commonly used labels to provide a detection signal in biological assays. Covalently attaching such a label to an antibody combines the unique specificity of the antibody with a sensitive means for detection, thus creating an ideal probe molecule.

The antibodies can be labeled in two different ways. First, the *direct method* is a one-step method in which a labeled antibody directly reacts with the antigen of interest. This direct detection method was first introduced in the 1940s by Coons et al. when they labeled antibodies with a fluorescent tag to mark tissue antigens (Coons et al., 1942).

Second, the *indirect method* is a two-step method in which an unlabeled primary antibody (first layer) reacts with the antigen, and a labeled secondary antibody (second layer) which reacts with the primary antibody. The second layer antibody is mostly labeled with a fluorescent dye or an enzyme. This indirect detection was first described by Weller and Coons (1954) and is still a popular method. The advantages and disadvantages of direct and indirect labeling methods are given in Table 3.

Few examples of biosensors fabricated using antibodies as the sensing element for the detection foodborne pathogens includes, surface plasmon resonance (SPR) (Taylor et al., 2006; Waswa et al., 2007), evanescent wave fiber-optic biosensors (Lim, 2003), nanowire labeled direct-charge transfer biosensor (Pal et al., 2007), self-excited PZT-glass microcantilevers (Campbell and Mutharasan, 2005), magnetoelastic resonance sensors (Guntupalli et al., 2007) and immunosensors (Tokarsky and Marshall, 2008).

4.2. Enzyme bioreceptors

Biosensors that make use of enzymes as the biorecognition elements are a well developed and widely studied area. Enzymes are chosen based on their specific binding capability and their catalytic activity and the chosen enzyme with a suitable substrate should provide sufficient electron transfer to the working electrode (Vo-Dinh and Cullum, 2000). Almost all enzymes are proteins with an exception of a few ribonucleoprotein enzymes, in which the catalytic activity is in the RNA rather than the protein part. In the field of pathogen detection, using enzymes as bioreceptors not only provides biosensors with a high degree of specificity, but their catalytic activity can amplify the pathogenic bacteria being detected and measured, allowing for sensitive analyses. In most of the cases enzymes are used to function as labels than the actual bioreceptor. Owing to the improvements in enzyme-labeling methods during the past decades, enzyme-labeled antigens and antibodies have been increasingly used.

The use of enzymes as labels has gained more popularity in immunoassay detection than other labeling methods such as radioisotope and fluorescent tag. Enzymes offer the advantages of high sensitivity, possibility of direct visualization and are stable for years. But there are some disadvantages found when using enzymes as labels, which include multiple assay steps and the possibility of

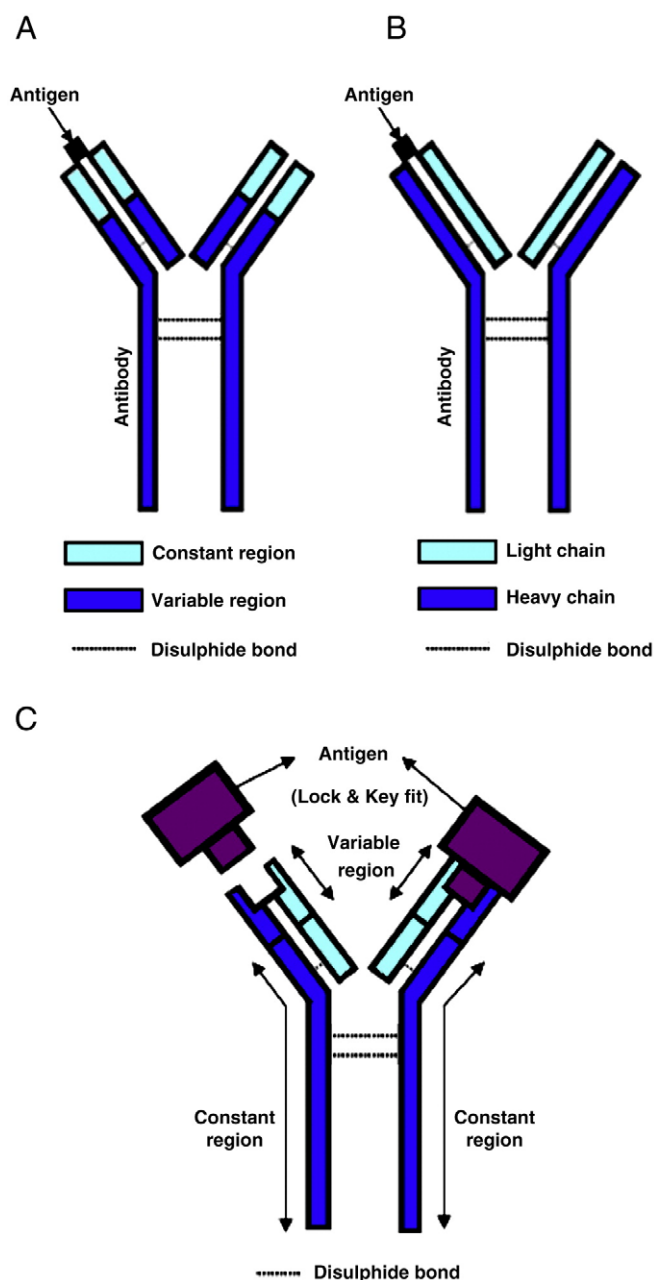


Fig. 4. (A) and (B): The basic structure of an antibody. (C): Antigen–antibody lock and key fit.

interference from endogenous enzymes. Using enzymes as labels offers several advantages over fluorescently labeled and radiolabeled substances. Enzyme immunoassay reagents are more stable, sensitive and there are no health hazards. Example, disposal of waste associated with radioisotopic labels. Many enzyme detection methods are visual, eliminating the need for expensive, complicated equipment. Antibodies/antibody fragments labeled to small enzymes can readily pass through cell membranes and by using light microscope it is then possible to localize and observe cellular antigens to tissue structures. Also, the tissue sections that have been developed with the appropriate substrate can be stable for years.

Whereas, in most immunofluorescent staining techniques, the fluorescent signal rapidly decreases due to exposure to light. There are three enzymes generally used with ELISA's. The earliest was alkaline phosphatase and the most commonly used now is horseradish peroxidase (HRP) and the third is beta-galactosidase. Many papers have been reported since the past 20 years, where immunosensor

Table 3

Comparison of direct and indirect antibody labeling methods.

Method	Advantages	Disadvantages
Direct	1. Simple and rapid method since only one antibody is used. 2. There is no cross-reaction since secondary antibody is eliminated.	1. Immunoreactivity of the primary antibody may be reduced as a result of labeling. 2. Time-consuming and expensive since each and every primary antibody is to be labeled. 3. Less sensitive and hence very small signal amplification.
Indirect	1. Immunoreactivity of the primary antibody is not affected. 2. Greater sensitivity allowing for signal amplification. 3. A wide variety of labeled secondary antibodies are available commercially.	1. Cross-reactivity may occur with the secondary antibody which results in nonspecific signal. 2. Procedure involves extra steps than the direct method.

systems used enzymes as labels, for the detection of pathogens in food. For example, a report discussing the applications of highly dispersed carbon particles which utilized sandwich immunoassay format used horseradish peroxidase (HRP) enzyme to label the antibody for the detection of pathogenic bacteria's such as *L. monocytogenes*, *E. coli* and *C. jejuni* (Chemburu et al., 2005).

4.3. Nucleic acid bioreceptors

Recent advances in nucleic acid recognition have enhanced the power of DNA (deoxyribonucleic acid) biosensors and biochips. In the case of nucleic acid bioreceptors for pathogen detection, the identification of a target analyte's nucleic acid is achieved by matching the complementary base pairs that are often the genetic components of an organism. Since each organism has unique DNA sequences, any self-replicating microorganism can be easily identified. Biosensors based on nucleic acid as biorecognition element are simple, rapid, and inexpensive and hence it is widely used in pathogen detection. In contrast to enzyme or antibodies bioreceptors, nucleic acid recognition layers can be readily synthesized and regenerated. DNA damage is one of the most important factors to be considered when nucleic acid bioreceptor are used. Hundreds of compounds bind and interact with DNA. Detection of chemicals may cause irreversible damage to DNA by changing the structure of DNA and the base sequence, which in turn disturbs the DNA replication. Due to their wide range of physical, chemical and biological activities, nucleic acid based biosensors have been reported by many researchers for the detection of food pathogen like *E. coli* O157:H7 (Chen et al., 2008), *Salmonella* spp. (Lermo et al., 2007), *Campylobacter jejuni* (Uyttendaele et al., 1997) etc. DNA hybridization microarrays have been suggested as a platform for the parallel detection of multiple pathogenic microorganisms in food in a relatively short time.

DNA microarray techniques have been reported for the simultaneous detection of food-borne pathogens including *Listeria* spp., *Campylobacter* spp., *S. aureus* enterotoxin genes and *Clostridium perfringens* toxin genes and their virulence factors (Sergeev et al., 2004). Using single-stranded nucleic acid aptamer, Kim invented a rapid method for specific identification and quantification of food-borne pathogens (Kim, 2007). The single-stranded nucleic acid aptamer specifically binding to a food-borne pathogen had a nucleotide selected sequence wherein the food-borne pathogens were *E. coli*, *Salmonella* spp., *Listeria* spp. and *Staphylococcus* spp.

Recent advances in nucleic acid recognition, like the introduction of peptide nucleic acid (PNA) has opened up exciting opportunities for DNA biosensors. PNA is a synthesized DNA in which the sugar–phosphate backbone is replaced with a pseudopeptide. PNA as a probe molecule has several advantages like superior hybridization characteristics, detection

of single-base mismatches and improved chemical and enzymatic stability relative to nucleic acids. In addition, its different molecular structure enables new modes of label-free detection contributing significantly towards the establishment of rapid, stable and more reliable analytical processes. PNA based nucleic acid recognition have been reported by many researchers (Briones et al., 2004; Fan et al., 2007; Li et al., 2001; Mateo-Marti et al., 2007; Sawata et al., 1999; Steichen et al., 2007). PNA sensing can be utilized to detect desired pathogens with high stability and increased affinity for target sequences. But the main drawback of PNA is that synthesis is very expensive. The other disadvantages of PNAs are also becoming evident. For example, long PNA oligomers, depending on their sequence, are prone to aggregation, difficult to purify and difficult to characterize. In addition, purine-rich PNA oligomers tend to aggregate and are poorly soluble in aqueous media (Gall et al., 2003).

4.4. Cellular bioreceptors

In cellular structures/cells based bioreceptors biorecognition is either based on whole cell/microorganism or a specific cellular component that is capable of specific binding to certain species. The cellular systems, enzymes and non-enzymatic proteins are the three major sub-classes of this category. Since the biosensors based on enzyme bioreceptors have been discussed previously on their own classification, biorecognition based on the other two sub-classes are presented here.

4.4.1. (i) Cellular systems

The ability of cells to recognize and respond to stimuli has made them attractive components for incorporation into biosensors (Pancrazio et al., 1999). In cell-based biosensors (CBB), a whole cell serves as the molecular recognition element and requires two transduction phases. The cells serve as the primary transducer, converting the detected analyte into a cellular response. A second transducer is required to convert the cellular signal into an electronic signal that can be processed and analyzed. The second transduction is dependent on the type of cellular signal to be monitored. There are many reasons why living cells are well-suited for recognition. First, cells provide sensitivity to a wide range of biochemical stimuli since they contain many highly evolved biochemical pathways. Secondly, cells provide a functional assay for biochemical agents. Because CBB make use of direct measurements of physiologic function (and changes induced by toxins), they provide detection capability for previously unknown agents as well. The third major advantage associated with cells as bioreceptors for incorporation into biosensors, is that the detection limits can be very low, because of signal amplification. The above properties distinguish CBB from molecular biosensors that rely on the detection of molecular events such as antibody binding, DNA hybridization, or enzymatic reactions.

Though the CBB were reported by many researchers for the identification and quantification of the pathogenicity induced by various foodborne pathogens, they have some major difficulties as well. Because, the cells need a specific environment to function normally and also, as they are subject to biological variability, the cells duplicate which leads to the reproducibility of sensor responses. Moreover, their self-life is very short and preservation is not easy. In addition to the above disadvantages, it is difficult to classify the type of agent based on functional activity of the cells, because multiple biochemical pathways can lead to the same cellular response. However, due to the development of genetically-engineered cell-based biosensor (GECBB) this has been overcome.

A new approach using a GECBB for functional classification of agents was described by Alexander (Aravanis et al., 2001). The GECBB operates by examining for an agent's ability to differentially activate two populations of cells, wild-type (WT) cells and cells genetically engineered to lack a specific receptor, knockout (KO) cells. Any

biological agent that targets the knocked out receptor will evoke a response in the WT but not in the KO. Thus, the GECBB is exquisitely sensitive to agents that affect the engineered pathway. This approach provides the benefits of an assay for specific functional activity while simplifying signal analysis. The use of GECBB for pathogen identification was reported by many researchers. Rider et al. (2003) demonstrated the use of genetically engineered B lymphocyte cells for a rapid identification of pathogens at very low levels. This sensor used B lymphocytes that have been engineered to emit light within seconds of exposure to specific bacteria and viruses. Because of its speed, sensitivity, and specificity, they suggested that this pathogen identification technology proves to be useful for food quality monitoring and other applications as well.

Biosensors incorporating mammalian cells to detect foodborne pathogens are drawing increasing interest because they respond in a manner that can offer insight into the physiological effect of an analyte. In addition, this type of cell-based biosensor has the unique potential of distinguishing between viable and nonviable bacterial cells, which is a critical determination for the food industry since nonviable bacterial cells in foods themselves are not considered to be potential threats (Bhunja et al., 2007). Recently, the first example of a cell-based sensing system using collagen-encapsulated mammalian cells for rapid detection of pathogenic bacteria or toxin was presented by Banerjee et al. (2008). A multi-well plate-based biosensor containing B-cell hybridoma, Ped-2E9, encapsulated in type I collagen matrix, was demonstrated for the rapid detection of viable cells of foodborne pathogens like *Listeria*, the toxin listeriolysin O, and the enterotoxin from *Bacillus* species. These pathogenic micro-organisms or toxins can infect and produce detectable cytotoxicity to Ped-2E9 cells, which release alkaline phosphatase when infected. It has been reported that, the pathogenic *L. monocytogenes* cells and toxin preparations from *L. monocytogenes* or *B. cereus* showed cytotoxicity ranging from 24 to 98% at 3–6 h postinfection. In contrast, nonpathogenic *L. innocua* (F4247) and *B. subtilis* induced minimal cytotoxicity, ranging only 0.4–7.6%.

Carlyle et al. (2002) demonstrated the application of chromatophore cell-based biosensor assay for the detection of foodborne and other microbial pathogens. Where, the chromatophores are pigmented cells derived from *Betta splendens* that undergo a vivid colour response, due to aggregation, when exposed to specific biologically active agents. The paper reported that, Chromatophore cells were exposed to purified bacterial toxins and live toxin producing bacterial strains to test the effect of bacterial toxins. Where, toxin producing strains of *E. coli*, *Salmonella*, *Shigella*, *Vibrio*, *B. cereus* and *Clostridium* induced aggregation of the pigmented organelles in the chromatophore cells and the non-toxin producing strains of *E. coli*, *Bacillus subtilis* and *Lactococcus lactis* did not induce aggregation. Therefore it indicates that the aggregation of the Chromatophore cells was toxin dependent and it is nonreversible.

Zhao et al. (2006) developed an artificial cell-based biosensor that used a liposome-doped silica nanocomposite, which mimics existing whole-cell assays for Listeriolysin O (LLO), a pore-forming hemolysin secreted by the foodborne pathogen *L. monocytogenes*. The liposomes were encapsulated in porous silica using alcohol-free sol-gel synthesis methods. The immobilized liposomes act as a cellular compartment containing the fluorescent dyes. The dye release due to the pore formation by LLO indicated the presence of the toxin. In contrast to the mammalian cell-based biosensors, the synthetic cell-based biosensors have very long self-life and preservation is easier, since these sensors are based on the “non-living” system.

4.4.2. (ii) Non-enzymatic proteins

Many proteins that are found within cells often serve the purpose of bioreception for intracellular reactions that will take place later or in another part of the cell (Vo-Dinh and Cullum, 2000). These proteins can be used for transport of a chemical from one place to another, such

as a carrier protein or channel protein on a cellular surface. In any case, these proteins provide a means of molecular recognition through one or another type of mechanism specifically, active site or potential sensitive site. These proteins can be attached to various types of transducers for bacterial pathogen detection.

Ertl et al. (2003) reported an electrochemical, screen-printed biosensor array, where selective recognition was accomplished using lectins that recognize and bind to cell-surface lipopolysaccharides. Screen-printed sensor arrays in conjunction with factor analysis were used for the rapid identification of four *E. coli* subspecies (*E. coli* B, *E. coli* Neotype, *E. coli* JM105 and *E. coli* HB101) and their results suggested that identification of *E. coli* subspecies is possible within 40 min. Earlier work of this group showed the application of lectins to the recognition of distinct surface lipopolysaccharide structures on a broad range of microbial strains (Ertl and Mikkelsen, 2001). The lectin-based sensor array was exposed to viable cells of Gram-negative and Gram-positive as well as yeast, and chronocoulometric and they used principal component analysis to classify the chronocoulometric results for the different microbial strains. With this type of biosensor, they were able to distinguish between six microbial species such as *B. cereus*, *S. aureus*, *Proteus vulgaris*, *E. coli*, *Enterobacter aerogenes* and *Saccharomyces cerevisiae* (Ertl and Mikkelsen, 2001).

4.5. Biomimetic receptors

A receptor that is fabricated and designed to mimic a bioreceptor (antibody, enzyme, cell or nucleic acids) is often termed a biomimetic receptor. Though there are several methods, such as genetically engineered molecules and artificial membrane fabrication, the molecular imprinting technique has emerged as an attractive and highly accepted tool for the development of artificial recognition agents. Molecular imprinting is one of the techniques of producing artificial recognition sites by forming a polymer around a molecule which can be used as a template. Molecular imprinted polymers (MIPs) can, in principle, be synthesized for any analyte molecule and are capable of binding target molecules with affinities and specificities on a par with biological recognition elements (Haupt and Mosbach, 2000). However, MIPs possess many disadvantages as well. For example, it is hard to completely remove the template from MIPs, the imprinted polymer is insoluble, and although the polymer contains many imprinted cavities, only some are really good and match the template molecule.

Using biomimetic polymer sensor, Silbert et al. (2006) presented a new platform for visual and spectroscopic detection of bacteria. The detection scheme in their study was based on the interaction of membrane-active compounds secreted by bacteria with agar-embedded nanoparticles comprising phospholipids and the chromatic polymer polydiacetylene (PDA). PDA undergoes dramatic visible blue-to-red transformations together with an intense fluorescence emission that are induced by molecules released by multiplying bacteria. The chromatic transitions were easily identified by the naked eye and they suggested that since the chromatic technology is generic and simple, eliminating the need for prior identification of specific bacterial recognition elements, this can be applied for detection of both Gram-negative and Gram-positive bacteria. The bacteria used in their study were *S. enterica*, *B. cereus*, and *E. coli*. But the draw back of their bacterial detection was that the assay was incapable to facilitate specificity of detection among bacterial species. However, they explained that the nonspecificity of the chromatic platform can benefit in evaluating food freshness where, the presence of any type of bacteria can be reported.

Ma et al. (1998), demonstrated that glycolipids could be inserted into the polydiacetylene matrix based on noncovalent binding, where the polymerized vesicles using 2,4-tricosadiynoic acid (2,4-TCDA) as the matrix lipid and dioctadecyl glyceryl ether- β -glucoside (DGG) as the receptor could make a biosensor to detect *E. coli*. A major limitation of this method was the extravagant sensitivity of 2, 4-TCDA and there was no colour change when *E. coli* was added into the

mixed PCDA/DGG vesicle solution. These obstacles have been overcome, when the same group of researchers reported that supramolecular assembly of phospholipid-polymerized diacetylene vesicles functionalized with glycolipid can provide a molecular recognition function (Su et al., 2005b). They demonstrated the fabrication of mixed phospholipid/polydiacetylene vesicles functionalized with glycolipid for the colorimetric detection of *E. coli*, where the direct coupling of a recognition event leads to a visible colour change from blue to red, readily seen with the naked eye and quantified by absorption spectroscopy.

Biochromic conjugated polymer (BCP) sensors are another type of biomimetic based biosensor for pathogen detection, demonstrated by Song et al. (2002). Biologically active cell membrane components were incorporated into conjugated polymers with desirable optical properties. Polydiacetylenic membrane-mimicking materials that mimic the cell membrane and conveniently report the presence of pathogens with a colour change were used for the colorimetric detection of bacterial toxins and influenza virus. It was suggested that the BCP sensors are convenient for microfabrication and use, since their molecular recognition and signal transduction functionalities are resident in a single functional unit (Song et al., 2002). Though many biomimetic-based biosensors for foodborne pathogen detection have been published in recent years, only very few applications to food samples have been reported to date.

4.6. Bacteriophages

From the past decades to today, enzymes, antibodies, nucleic acids and biomimetic materials are used as biomolecular recognition elements and they have both merits and demerits when compared to one another. Recently, bacteriophages are employed as biorecognition elements for the identification of various pathogenic micro organisms. These powerful bacteriophages (phages) are viruses that bind to specific receptors on the bacterial surface in order to inject their genetic material inside the bacteria. These entities are typically of 20–200 nm in size (Singh et al., 2009). Phages recognize the bacterial receptors through its tail spike proteins. Since the recognition is highly specific it can be used for the typing of bacteria and hence opened the path for the development of specific pathogen detection technologies. Researchers have reported the application of phage as a biorecognition element for the detection of various pathogens such as *E. coli* (Singh et al., 2009), *S. aureus* (Balasubramanian et al., 2007) and *B. anthracis* spores (Huang et al., 2008; Xie et al., 2009) by using different sensing platforms.

5. Transducers

The transducer plays an important role in the detection process of a biosensor. Biosensors can also be classified based upon the transduction methods they employ. Wide varieties of transduction methods have been developed in the past decade for the detection of foodborne pathogens. Although there are new types of transducers constantly being developed for use in biosensors, the transduction methods such as optical, electrochemical and mass based are given importance here since these are the most popular and common methods. Each of these three main classes contains many different subclasses and they can be further divided into label and label-free (non-labeled) methods. Where, the labeled methods depend on the detection of a specific label and the label-free detection is based on the direct measurement of a phenomenon occurring during the biochemical reactions on a transducer surface.

5.1. Optical-based biosensors

Optical biosensors have received considerable interest for bacterial pathogen detection due to their sensitivity and selectivity. Optical-

based detection offers large number of subclasses based on absorption, reflection, refraction, dispersion, infrared, Raman, chemiluminescence, fluorescence, and phosphorescence. However, all the above subclasses require a suitable spectrometer to record the spectrochemical properties of the analyte. The most commonly employed techniques of optical detection are surface plasmon resonance and fluorescence due to their sensitivity. Optical techniques using fiberoptics, laser, prism and waveguides are also employed for pathogen detection.

5.1.1. Raman and Fourier transform infrared spectroscopy

Vibrational spectroscopies such as Raman scattering and Fourier transform infrared (FT-IR) are the more frequently reported whole-organism fingerprinting techniques. However, before any of these whole-organism fingerprinting techniques can be used to analyze sample of interest, the microorganisms must be cultured in order to isolate the analyte from other sample constituents and/or produce sufficient biomass for analysis.

Raman spectroscopy, an optical technique based on light scattering, has been investigated by many researchers as a means of rapid detection of bacterial pathogens. Schmilovitch et al. (2005) employed a dispersive system spectrophotometer, with a 785 nm diode laser to detect the presence of Gram-positive and Gram-negative bacteria. A clear distinction between samples containing bacteria and clean samples were obtained by this method. Also the system was able to determine bacterial concentrations within 2% of the level in the basic bacteria suspension. During the past decade, surface-enhanced spectroscopy technique based on employing the phenomenon that can enhance the normal signal by many orders of magnitude was developed to eliminate the need for culturing. In this approach the pathogens of interest are selectively isolated from the sample using capture biomolecules, which add an additional layer of specificity to the whole-organism fingerprinting, and the analysis is exceptionally specific, making it possible to differentiate among species/strains that cross-react with a given biomolecule.

Grow et al. (2003) developed a detection method that uses surface-enhanced Raman scattering microscopy for label-free transduction (SERS). Their initial studies have shown that the Gram-positive *Listeria* and Gram-negative *Legionella* bacteria, *Bacillus spores* and *Cryptosporidium oocysts* can often be identified at the subspecies/strain level on the basis of SERS fingerprints collected from single organisms. Therefore they demonstrated that pathogens can be individually identified by μ SERS, even when organisms that cross-react with the capture biomolecules are present in a sample. Moreover, the SERS fingerprint reflects the physiological state of a bacterial cell, e.g., when pathogenic *Listeria* and *Legionella* were cultured under conditions known to affect virulence, their SERS fingerprints changed significantly (Grow et al., 2003).

Another report by Goeller and Riley (2007) demonstrated the analysis of pathogen detection using Raman spectroscopy without drying the microorganisms. Confocal Raman measurements of the bacterium *E. coli* and of two bacteriophages, MS2 and PRDI, were analyzed for characteristic peaks and to estimate detection limits using traditional Raman and SERS. With the obtained results they demonstrated that both Raman spectroscopy and SERS have a potential as a pathogen monitoring platform. Also Raman spectroscopy can be combined with other optical methods for pathogen detection. Recently, Kalasinsky et al. (2007) reported Raman chemical imaging spectroscopy (RCIS), which combines Raman spectroscopy, fluorescence spectroscopy, and digital imaging. Using this method, trace levels of pathogens were detected without the use of amplification or enhancement techniques.

Fourier transform infrared (FT-IR) spectroscopy is a non-destructive analytical technique with considerable potential for application in the foodborne pathogen detection. FT-IR spectrometry-based approach was developed by Yu et al. (2004a) for microbial differentiation and

quantification of eight different microorganisms including *Salmonella*. FT-IR spectroscopy combined with chemometrics was able to differentiate the microorganisms studied at low concentration level of 10^3 colony-forming units (CFU)/mL in apple juice. Another report demonstrated the use of FT-IR spectrometry to differentiate *E. coli* O157: H7 from other bacteria inoculated into apple juice (10^9 CFU/mL) (Al-Holy et al., 2006). Lin et al. (2004) used FT-IR spectrometry to differentiate between intact and injured *Listeria* and to distinguish this strain from other selected *Listeria* strains.

Also it has been reported by Ellis et al. (2002) that FT-IR technique can be used directly on the surface of food to produce biochemically interpretable “fingerprints”. FT-IR was exploited to measure biochemical changes within the meat substrate, enhancing and accelerating the detection of microbial spoilage. Quantitative interpretation of FT-IR spectra was possible using partial least-squares regression and allowed accurate estimates of bacterial loads to be calculated directly from the meat surface in 60 s. The same group of researchers recently reported the qualitative interpretation of FT-IR and Raman spectra for the identification and authentication of muscle foods (Ellis et al., 2005).

5.1.2. Surface plasmon resonance

A common method which uses reflectance spectroscopy for the pathogen detection is surface plasmon resonance (SPR). SPR is able to detect minor changes in the refractive index, which occur when cells binds to receptors immobilized on the transducer surface and it measures the change of the angle of the reflected light as a function of change of density of medium against time. Direct label-free detection of pathogens is also possible using this method. SPR based biosensors have been reported by many researchers for the detection of foodborne pathogens such as *L. monocytogenes* (Bhunja et al., 2004; Koubova et al., 2001; Taylor et al., 2006), *Salmonella* (Bhunja et al., 2004; Koubova et al., 2001; Oh et al., 2004; Taylor et al., 2006), *E. coli* O157:H7 (Meeusen et al., 2005; Oh et al., 2003; Subramanian et al., 2006; Taylor et al., 2006; Taylor et al., 2005; Waswa et al., 2007), and *C. jejuni* (Taylor et al., 2006). Also, commercially available optical biosensors that use SPR for monitoring and identifying pathogens were employed by many researchers for the detection of foodborne pathogens. Spreeta™ biosensor was used for the detection of *E. coli* O157: H7 (Meeusen et al., 2005; Waswa et al., 2007); BIACORE 3000 was used for the detection of *L. monocytogenes* (Leonard et al., 2004), and *Salmonella* (Bokken et al., 2003).

Recently, Waswa et al. (2007) demonstrated the use of the Spreeta (TM), SPR-based biosensor for the detection *E. coli* O157:H7. In the Spreeta™ biosensor light from an LED is reflected off a gold surface, and the angle and intensity corresponding to the SPR minimum is measured and represented as a refractive index change corresponding to the antigen–antibody coupling at the sensor surface. They conducted the assay at near real-time and results were obtained after about 30 min of sample injection. Sensitivity of the *E. coli* O157: H7 assay was 10^2 – 10^3 CFU/mL. The biosensor assay was also specific to *E. coli* O157:H7 as other organisms (*E. coli* K12 and *Shigella*) did not produce any appreciable change in the sensogram. The paper suggested that further experiments are needed to establish well-defined methods for detecting other food-borne pathogens in more complex and specific food matrices. Also, Wei et al. (2007) used the Spreeta™, SPR-based biosensor for the rapid identification of *C. jejuni*. Balasubramanian et al. (2007) reported the label-free detection of *S. aureus* using lytic phage as highly specific and selective biorecognition element and surface plasmon resonance-based SPREETA™ sensor as a detection platform. Lytic phage was immobilized on the gold surface of SPREETA sensor via trouble-free direct physical adsorption. The detection limit was found to be 10^4 CFU/mL. The paper reported that the preliminary results using lytic phage as a probe for bacterial detection, in combination with SPR platform were promising and hence can be employed for rapid and label-free detection of different bacterial pathogens.

A successful biosensor must distinguish a target analyte from a complex mixture. The work by Taylor et al. (2006) demonstrated the use of multi-channel SPR biosensor for the simultaneous discrimination of multiple target analytes from complex mixtures. A custom-built SPR sensor based on the Kretschmann geometry of the attenuated total reflection (ATR) method and wavelength modulation was used. The quantitative and simultaneous detection of the four species of bacteria, *E. coli* O157:H7, *Salmonella choleraesuis* serotype typhimurium, *L. monocytogenes*, and *C. jejuni*, using an eight-channel surface plasmon resonance (SPR) sensor based on wavelength division multiplexing was reported. Amplification using secondary antibodies allowed to achieve a lower detection limit, while assuring specific detection. The experiments, demonstrated the specificity of these secondary antibodies, which were tested after the exposure of a sensor surface to three non-target species of bacteria at concentrations of 10^7 CFU/mL (Taylor et al., 2006). The limit of detection for each of the four species of bacteria in the tested matrices ranged from 3.4×10^3 to 1.2×10^5 CFU/mL and the detection curves in buffer of individual bacteria in a mixture of all four species of bacteria correlated well with detection curves of the individual species of bacteria alone. The results suggested that specific detection of pathogens in a complex mixture of different species of bacteria can be achieved using secondary antibody amplification.

SPR-based immunosensor have been reported for the detection of foodborne pathogens (Oh et al., 2004; 2003; Subramanian et al., 2006). Self-assembled monolayers (SAM) based SPR immunosensor for the rapid and specific detection of *E. coli* O157:H7, using a sandwich assay has been reported by Subramanian et al. (2006). The paper investigated the effect of protein G based detection and the effect of concentrations of primary and secondary antibodies in sandwich assay. The sensor could detect as low as 10^3 CFU/mL of *E. coli* O157:H7 in a sandwich assay, with high specificity against *S. enteritidis*. The detection limit using direct assay and protein G were 10^6 CFU/mL and 10^4 CFU/mL, respectively. Their results indicated that an alkanethiol SAM based SPR biosensor has the potential for rapid and specific detection of *E. coli* O157:H7, using a sandwich assay. Also, SPR-based immunosensor using self-assembled protein G for the detection of *Salmonella paratyphi* with a detection range of 10^2 – 10^7 CFU/mL has been reported by Oh et al. (2004). Previous work by the same group of researchers reported SPR-based immunosensors using self-assembled protein G for the detection of *E. coli* O157: H7 with detection limit of 102 CFU/mL (Oh et al., 2003).

5.1.3. Optical fibers

Based on the principle of total internal reflection (TIR), optical fibers transmit light. The fiber optic biosensor uses tapered fiber to send excitation laser light to the detection surface and receive emitted light. Propagation of light through a fiber or waveguide can be very sensitive to the surroundings, which makes the optical fibers excellent detectors for a variety of applications in foods such as identification and detection of pathogens. Also, it was highlighted that the detection limits of fiber optical biosensors are comparable to the sophisticated large bench-top instruments (Monk and Walt, 2004). The use of fiber optic biosensors and the combination of optical fiber with other optical techniques for the detection of foodborne pathogens, such as *Listeria* (Strachan and Gray, 1995), *Salmonella* (Ko and Grant, 2006; Morgan et al., 2006), *E. coli* O157:H7 (DeMarco and Lim, 2002; Simpson and Lim, 2005; Ye et al., 2002) and *Clostridium-botulinum* toxin (Ogert et al., 1992) were reported.

A fiber optic portable biosensor utilizing the principle of fluorescence resonance energy transfer (FRET) for fast detection of *Salmonella typhimurium* in ground pork samples was reported (Ko and Grant, 2006). A portable evanescent-wave fiber-optic biosensor to detect *E. coli* O157:H7 in seeded 10-g and 25-g ground beef samples was demonstrated by (DeMarco and Lim, 2002). A centrifugation method to obtain samples suitable for biosensor analysis from 10-g

and 25-g ground beef samples was developed. The paper reported that the assay was sensitive and repeatable. One hundred percent correct identification of positive samples was obtained at 9.0×10^3 CFU/g for 25-g ground beef samples with silica waveguides and at 5.2×10^2 CFU/g for 10-g ground beef samples with polystyrene waveguides. With the obtained results they demonstrated that the reaction were highly specific.

The fiber-optic biosensors can also be used to reduce the impact of PCR inhibitors present in complex matrices in food. Though the fiber-optic biosensors can rapidly detect pathogens from complex matrices, confirmation tests may take up to several hours to complete. Simpson and Lim (2005) presented the methodologies to reduce the time necessary for confirmation from 10 to about 2 h in the detection of *E. coli* O157:H7 by direct PCR of bacteria from the fiber-optic waveguides without the need for culture or enrichment steps. A fiber-optic surface plasmon resonance biosensor for detection of Staphylococcal enterotoxin B (SEB) was reported (Slavik et al., 2002). For specific detection of SEB, the fiber-optic SPR sensor is functionalized with a covalently crosslinked double-layer of antibodies against SEB. It has been demonstrated that the fiber-optic SPR biosensor was able to detect ng/mL concentrations of SEB in less than 10 min.

Ye et al. (2002) developed a biosensor, consisting of a chemiluminescence reaction cell, a fiber-optic light guide, and a luminometer linked to a PC, in conjunction with immunomagnetic separation for rapid detection of *E. coli* O157:H7. The cell number of *E. coli* O157:H7 was determined by collecting the HRP-catalyzed chemiluminescence signal from the bead surface through a fiber-optic light guide and measuring the signal with a luminometer. The chemiluminescence biosensor was selective to *E. coli* O157:H7 in the presence of other bacteria in the sample, including *S. typhimurium*, *C. jejuni*, and *L. monocytogenes*. The detection limit of the biosensor was 1.8×10^2 CFU/mL within 1.5 h. By using the above method the same group of researchers demonstrated the detection of *E. coli* O157:H7 in ground beef, chicken carcass, and lettuce samples with the detection limits of 3.2×10^2 , 4.4×10^2 , and 5.5×10^2 CFU/mL, respectively and the detection was completed within 1.5 h without any enrichment (Liu et al., 2003). A fiber-optic immunosensor for the rapid and specific detection of *S. aureus* has been reported (Chang et al., 1996). A sensitive fiber-optic that produces evanescent waves was developed for the detection of protein A, a product secreted only by *S. aureus*. The principle of the detection involved a sandwich immunoassay with fluorescein isothiocyanate conjugated with anti-(protein A) immunoglobulin G to produce signals of the antigen–antibody reaction, and the detection limit obtained was 1 ng of protein A per millilitre. Also, commercially available fiber-optic biosensors have been used for food pathogen detection. RAPTOR, a portable and automated fiber-optic biosensor was used to detect *S. enteritidis* in food samples (Morgan et al., 2006).

5.1.4. Other optical techniques

Recently, Ramachandran et al. (2008) demonstrated the use of optical micro-ring resonators as a platform for quantitative and qualitative biosensing applications. Vertically coupled, high refractive index micro-ring resonators were used as sensing elements, fabricated on silicon chips by photolithographic techniques. An optical reader system consisting of a near-infrared broad band light source and an optical spectrum analyzer was employed for data acquisition. They modified the micro-ring resonator surfaces with specific target receptors, including antibodies and single-stranded DNA oligonucleotides. Also, the paper suggested that the system can be successfully used for label-free, specific, and rapid detection of whole bacterial cells, proteins and nucleic acids (Ramachandran et al., 2008). The use of terahertz time domain spectroscopy for determination of ligand binding for biomolecules has been reported by Markelz et al. (2004). Various optical-based techniques which were not discussed here are given in the Table 4.

Table 4

Different optical techniques employed for pathogen detection.

Optical techniques	Detected pathogen	Limit of detection	Assay time	References
Optic interferometer	<i>S. typhimurium</i>	10 ⁵ CFU/mL	12 h	(Seo et al., 1999)
Resonant mirror detection	<i>E. coli</i>			(Kiba et al., 2006)
Evanescent wave	<i>E. coli</i> O1 57:H7	3 to 30 CFU/mL	4 h	(Varkey et al., 2002)
Turbidimetry (based on the optical density measurements)	<i>E. coli</i> O1 57:H7, <i>Staphylococcus aureus</i> and <i>Y. enterocolitica</i>			(Barco Alcala et al., 2000)
Automated optical method	<i>Salmonella</i> spp. and <i>L.monocytogenes</i>	10–50 cells each in 25 g sample	24 h	(Peng and Shelef, 2001)
Diffuse reflectance spectroscopy	<i>E. coli</i> O157:H7	5×10 ⁵ to 5×10 ⁸ cells/mL	45 min	(Rahman et al., 2006)
Optical method using laser beam	<i>E. coli</i>	45 cells		(Acharya et al., 2006)
Surface-enhanced infrared absorption spectroscopy	<i>Salmonella</i>			(Brown et al., 1998; Seelenbinder et al., 1999)
Bidiffractive grating biosensor (BDG)	<i>Staphylococcus aureus</i> enterotoxin B, <i>Clostridium botulinum</i> , ricin toxin and <i>Francisella tularensis</i>			(O'Brien et al., 2000)
Near-infrared spectroscopy	Septicemia/toxemia (septoX) in chickens			(Dey et al., 2003)
Imaging ellipsometry (IE)	<i>E. coli</i> O157:H7, <i>S. typhimurium</i> , <i>Y. enterocolitica</i> , and <i>Legionella pneumophila</i>	10 ³ to 10 ⁷ CFU/mL		(Choi and Oh, 2008)
Quantum dots	<i>E. coli</i> O157:H7	10 ⁶ cells/mL		(Hahn et al., 2008)
Fluorescence microscopy	<i>E. coli</i> O157:H7, <i>S. typhimurium</i> , <i>Y. enterocolitica</i> , and <i>Legionella pneumophila</i>	10 ² CFU/mL		(Choi and Oh, 2008)
Chemiluminescence	<i>E. coli</i> O157:H7	10 ² to 10 ⁵ CFU/mL	30 min	(Mathew et al., 2004)
Chemiluminescence enzyme immunoassay	<i>E. coli</i> O157:H7	10 ¹ to 10 ² /g	24 h	(Kovacs and Rasky, 2001)
Chemiluminescent immunoassay	<i>E. O157:H7</i> , <i>Y. enterocolitica</i> , <i>S. typhimurium</i> , and <i>L. monocytogenes</i>	10 ⁴ to 10 ⁵ CFU/mL for all bacterial species		(Magliulo et al., 2007)
Bioluminescence	<i>E. coli</i>	<10 ³ cells	1 and 2 h resp.	(Frank et al., 2007c; Lehtinen et al., 2006)
	<i>E. coli</i> and <i>Salmonella</i>			(Blasco et al., 1998)
Bacteriophage-based bioluminescence	<i>E. coli</i> O157:H7	10 CFU/mL	4 h	(Brigati et al., 2007)
	<i>Salmonella</i>	10 ⁸ CFU/mL	1–3 h	(Chen and Griffiths, 1996)

5.2. Electrochemical biosensors

Electrochemical based detection methods are another possible means of transduction that has been used for identification and quantification of foodborne pathogens. Electrochemical biosensors can be classified into amperometric, potentiometric, impedimetric and conductometric, based on the observed parameters such as current, potential, impedance and conductance respectively. Different modes of electrochemical based foodborne pathogen detection are given in

Table 5. Although the electrochemical detection has several advantages like low cost, ability to work with turbid samples and easy miniaturisation, their sensitivity and selectivity are slightly limited when compared to optical detection. However, electrochemical biosensors were found coupled with other biosensing techniques for enhanced detection.

5.2.1. Amperometric method

Amperometric transduction is the most common electrochemical detection method which has been used for pathogen detection and it

Table 5

Different modes of electrochemical based foodborne pathogen detection.

Mode of detection	Analyte	Limit of detection (CFU/mL)	Assay time	References
Amperometric	<i>E. coli</i> O157:H7	81	6 min	(Muhammad-Tahir and Alocilja, 2004)
Amperometric	<i>E. coli</i> O157:H7	7.8×10 ¹	10 min	(Muhammad-Tahir and Alocilja, 2003b)
Amperometric	<i>E. coli</i> O157:H7	6×10 ² cells/mL	2 h	(Ruan et al., 2002)
Amperometric	<i>S. typhimurium</i>	1.09×10 ³	2.5 h	(Yang et al., 2001)
Amperometric	<i>Salmonella</i>	1–5	6 h	(Brooks et al., 1992)
Amperometric	<i>E. coli</i> O157:H7 without any enrichment after enrichment	1.6×10 ¹ –7.2×10 ⁷ 8.0×10 ⁰ –8.0×10 ¹	15 min 6 h	(Varshney et al., 2005)
Amperometric	<i>E. coli</i>	50 cells/mL	30 min overall	(Chemburu et al., 2005)
	<i>L. monocytogenes</i> , and <i>C. jejuni</i>	10 cells/mL 50 cells/mL		
Amperometric	<i>S. typhimurium</i>	5×10 ³ cells/mL	2 h	(Che et al., 1999)
Amperometric	<i>E. coli</i> O157:H7	50 cells/mL	35 min overall	(Abdel-Hamid et al., 1999b)
	<i>Salmonella</i>	50 cells/mL		
Amperometric	<i>E. coli</i> O157:H7	100 cells/mL	30 min	(Abdel-Hamid et al., 1999a)
Potentiometric	<i>E. coli</i> O157:H7 heat-killed	7.1×10 ² cells/mL	45 min	(Gehring et al., 1998)
	Live	2.5×10 ⁴ cells/mL	30 min	
Potentiometric	<i>E. coli</i>	10 cells/mL	1.5 h	(Ercole et al., 2003a)
Cyclic voltammetry	<i>V. parahaemolyticus</i>	7.374×10 ⁴		(Zhao et al., 2007)
Conductometric	<i>E. coli</i> O157:H7 and <i>Salmonella</i> spp.	7.9×10 ¹	10 min	(Muhammad-Tahir and Alocilja, 2003a)
Conductometric	<i>Bacillus cereus</i>	35–88	6 min	(Pal et al., 2008)
Impedimetric	<i>S. typhimurium</i>	4.8 and 5.4×10 ⁵	9.3 and 2.2 h respectively.	(Yang et al., 2004)
Impedimetric	<i>L. monocytogenes</i>			(Susmel et al., 2003; Tully et al., 2008)
	<i>Bacillus cereus</i>			(Susmel et al., 2003)
Impedimetric	<i>E. coli</i>	10 ¹ to 10 ⁷		(Munoz-Berbel et al., 2008)

has superior sensitivity than potentiometric method. In amperometric based detection the sensor potential is set at a value where the analyte produces current. Thus, the applied potential serves as the driving force for the electron transfer reaction, and the current produced is a direct measure of the rate of electron transfer. Clark and Lyons, (1962) reported that oxygen electrodes, possibly represent the basis for the simplest forms of amperometric biosensors, in which current was produced in proportion to the oxygen concentration. Many researchers have reported amperometric detection of foodborne pathogens such as *E. coli* O157:H7 (Abdel-Hamid et al., 1999a,b; Chemburu et al., 2005; Ruan et al., 2002; Varshney et al., 2005), *Salmonella* (Abdel-Hamid et al., 1999b; Brooks et al., 1992; Che et al., 1999; Yang et al., 2001), *L. monocytogenes* (Chemburu et al., 2005; Crowley et al., 1999) and *C. jejuni* (Chemburu et al., 2005).

Reymond et al. (2007) developed an amperometric detection method for determining the presence, the amount, and/or the concentration of an analyte in a microfluidic sensor, where the sensor is a part of an integrated sample acquisition system and/or analyte measurement device. Microfluidic sensor was filled with sample to be analyzed, and amperometry was performed to detect the analyte by applying the potential required to directly or indirectly detect the analyte. The method was adapted to detect the presence, amount and/or concentration of analytes. Various analytes are detected by oxidation and/or reduction using different applied potentials. In the method, the potential was applied and the related current measured at the integrated working electrode for no more than 10 s, nearly 2 s and a relaxation time separating sequential amperometric measurements was longer than 1 s but shorter than 1 min.

In order to detect aflatoxin M1 in milk, Micheli et al. (2005) compared two pathogen detection procedures for better detection and shorter analysis. The first procedure involved spectrophotometric enzyme-linked immunosorbent assay (ELISA), which exhibited a working range between 30 and 160 ppt in a direct competitive format. Then electrochemical immunosensors were fabricated by immobilizing the antibodies directly on the surface of the screen-printed electrodes. The electrochemical technique chosen was the chronoamperometry, performed at -100 mV. Results showed that using screen-printed electrodes, aflatoxin M1 was measured with a detection limit of 25 ppt and with a working range between 30 and 160 ppt (Micheli et al., 2005). A comparison between the spectrophotometric and electrochemical procedure showed that a better detection limit and shorter analysis time could be achieved using electrochemical detection. Electrochemical biosensors based on amperometric detection were found coupled with other biosensing techniques. For example, a bienzyme electrochemical biosensor was found coupled with immunomagnetic separation technique for the detection of *E. coli* O157:H7 (Ruan et al., 2002), *S. typhimurium* (Yang et al., 2001).

5.2.2. Potentiometric detection

In potentiometric based detection the bio-recognition process is converted into a potential signal. Usually a high impedance voltmeter is used to measure the electrical potential difference or electromotive force (EMF) between two electrodes at near zero current. Since potentiometry generates a logarithmic concentration response, the technique allows the detection of extremely small concentration changes. Not many potentiometric biosensors were found for the detection of pathogens. Light-addressable potentiometric sensor (LAPS) for the detection of pathogens has been reported (Ercole et al., 2003a; Gehring et al., 1998; Mackay et al., 1991). LAPS is based on the coupling of a transient photocurrent to an insulated *n*- or *p*-doped silicon thin layer in contact with an electrolyte. This transient photocurrent is induced by the application of transient illumination by using an intensity modulated light source like, light emitting diodes (LEDs). The magnitude of the induced photocurrent depends on the applied potential to the silicon plate. Also it is possible to detect different physicochemical phenomena by using different light sources

on different spatial regions. The control of several different parameters on a single device is possible if these regions differ structurally.

Ercole et al. (2003b) demonstrated the detection of *E. coli* cells in vegetable food using the potentiometric alternating biosensing (PAB) system based on LAPS. LAPS was used as a transducing element, for detecting pH variations due to NH_3 production by a urease-*E. coli* antibody conjugate. The liquid phase was analyzed both by PAB system and conventional CFU methods. The paper reported, the PAB system appeared to be very sensitive and fast, in comparison with conventional methods and the concentration of 10 cells/mL was detected in an assay time of ca. 1.5 h, which was from 10 to 20 times shorter than the conventional CFU procedure. Gehring et al. (1998) developed an immunoligand assay (ILA) in conjunction with a light-addressable potentiometric sensor (LAPS) for the rapid detection of *E. coli* O157:H7 cells in buffered saline. The ILA protocol consists of “sandwiching” bacterial analyte between biotinylated and fluoresceinated antibodies. Using ILA with LAPS, a minimum detectable level of $ca. 7.1 \times 10^2$ cells/mL of heat-killed or $ca. 2.5 \times 10^4$ cells/mL of Live *E. coli* O157:H7 bacteria were achieved in Tris-buffered saline in an assay time of ca.45 or ca.30 min, respectively.

5.2.3. Impedimetric detection

The integration of impedance with biological recognition technology for detection of pathogens has led to the development of impedance biosensors that are finding wide-spread use in the recent years (Yang and Bashir, 2008). Impedimetric transduction technique has been applied to detect and/or quantify variety of foodborne pathogens. Electrochemical impedance spectroscopy (EIS) is playing an important role in the biosensor development. In EIS measurements, a controlled AC electrical stimulus of between 5 and 10 mV is applied over a range of frequencies, and this causes a current to flow through the biosensor, depending on different processes. EIS is a widely used technique for probing bioaffinity interactions at the surfaces of electrically conducting polymers and can be employed to investigate ‘label-free’ detection of analytes via impedimetric transduction (Tully et al., 2008). Though, EIS offers label-free detection compared to amperometry or potentiometry, its detection limits are inferior compared to traditional methods.

Yang et al. (2004) used interdigitated microelectrodes as impedance sensors for rapid detection of viable *Salmonella*. The impedance growth curves, impedance against bacterial growth time, were recorded at four frequencies (10 Hz, 100 Hz, 1 kHz, and 10 kHz) during the growth of *S. typhimurium*. It was observed that impedance did not change until the cell number reached 10^5 – 10^6 CFU/mL. The greatest change in impedance was observed at 10 Hz. An equivalent electrical circuit, consisting of double layer capacitors, a dielectric capacitor, and a medium resistor, was introduced and used for interpreting the change in impedance during bacterial growth. Bacterial attachment to the electrode surface was observed with scanning electron microscopy, and it had an effect on the impedance measurement. The paper reported that the detection times for 4.8×10^5 and 5.4×10^5 CFU/mL initial cell numbers were 9.3 and 2.2 h, respectively (Yang et al., 2004).

5.2.4. Conductometric detection

Conductometric-based biosensors bond the relationship between conductance and a bio-recognition technique. Most reactions involve a change in the ionic species concentration, which leads to a change in electrical conductivity or current flow. Normally, a conductometric biosensor consists of two metal electrodes separated by a certain distance and an AC voltage applied across the electrodes causes a current flow. During a bio-recognition event the ionic composition changes and the change in conductance between the metal electrodes are measured.

Muhammad-Tahir and Alocilja (2003a) developed a conductometric biosensor for detecting foodborne pathogens such as *E. coli*

O157:H7 and *Salmonella* spp. It has been reported that, the lower limit of detection was approximately 7.9×10^1 CFU/mL within a 10 minute process and the biosensor provided specific, sensitive, low volume, and near real-time detection mechanism. Recently, Pal et al. (2008) developed a direct-charge transfer conductometric biosensor for the detection of *B. cereus* in various food samples. The biosensor used the principle of a sandwich immunoassay, combined with an electron charge flow aided through conductive polyaniline, to generate an electronic signal. The biosensor was able to detect cell concentrations in the range of 35–88 CFU/mL in the food samples with a detection time of 6 min. The biosensor was found to be specific to the target pathogen in pure cultures of *Bacillus megaterium* and generic *E. coli*.

Also it has been reported that the speed, sensitivity and ease of use, of this biosensor make it a promising device for rapid field-based diagnosis toward the protection of the food supply chain.

5.3. Mass-sensitive biosensors

Mass-sensitive biosensors are suitable for very sensitive detection, in which the transduction is based on the small changes in mass. The principal means of mass analysis depends on the use of piezoelectric crystals, which can be made to vibrate at a specific frequency with the application of an electrical signal of a specific frequency. The frequency of oscillation is therefore dependent on the applied electrical frequency to the crystal as well as the mass of the crystal. Therefore, when the mass increases due to binding of chemicals, the frequency of oscillation of the crystal changes and the resulting change can be measured electrically which can be used to determine the additional mass of the crystal. Quartz is being used as a common piezoelectric material. The two main types of mass based sensors are (1) bulk wave (BW) or quartz crystal microbalance (QCM) and (2) surface acoustic wave (SAW).

When a piezoelectric sensor surface, which has been coated with an antibody, is placed in a solution containing pathogens, the attachment of the agent to the antibody coated surface results in an increase in the crystal mass, and this gives rise to a corresponding frequency shift. Mass based detection is relatively easy to use, cost effective, and offers direct label-free analysis with increased sensitivity and specificity.

But foodborne pathogen detection based on piezoelectric sensor found to be very less compared to electrochemical and optical-based biosensors. Su and Li (2005) demonstrated a quartz crystal microbalance (QCM) immunosensor for the detection of *S. typhimurium* with simultaneous measurements of the resonant frequency and motional resistance. The immunosensor was fabricated using protein A for the antibody immobilization. High-frequency impedance analysis indicated that the changes in resonant frequency and motional resistance of the QCM were significant while the changes in static capacitance, motional capacitance, and motional inductance were insignificant. In the direct detection of *S. typhimurium* in chicken meat sample, resonant frequency and motional resistance were proportional to the cell concentration in the range of 10^5 – 10^8 and 10^6 – 10^8 cells/mL, respectively. The detection limit was lowered to 10^2 cells/mL by using anti-*Salmonella*-magnetic beads; however, motional resistance was not related to the cell concentration.

QCM immunosensor for the detection of *L. monocytogenes* with a detection limit of 1×10^7 cells/mL was reported (Vaughan et al., 2001). Also rapid (80 s) detection of *S. typhimurium* (Chen and Barbaree, 2002), *E. coli* O157:H7 (Berkenpas et al., 2006) with a surface acoustic wave (SAW) sensor has been reported. Nanduri et al. (2007) developed a biosensor based on landscape phages immobilized by physical adsorption on the surface of a QCM for detection of β -galactosidase from *E. coli* and reported that phage can be used as a recognition element in biosensors.

Mutharasan and Campbell (2008) reported a sensor flow cell for receiving a piezoelectric-excited millimeter-sized cantilever (PEMC)

sensor, which comprises a reservoir portion provided with a flow inlet aperture and a flow outlet aperture to allow a medium to flow into and out of the reservoir portion. The reservoir portion contains a sensing surface of the PEMC sensor, and at least a portion of the medium (liquid and/or a gas) exposable to the sensing surface. The method is found to be advantageous because, the exposure of the medium to the sensing surface of PEMC sensor is enhanced under the flow conditions as compared to a static medium. Thus, the ability of the sensor to detect the target material or analyte in the medium is superior under the flow conditions, as compared to stagnant conditions. The flow of medium enhances binding of analytes to the PEMC sensor surface, allowing detection of small concentration of analytes. It has been reported that, this method can be utilized for several biosensing applications including the detection of foodborne pathogens.

Recently, magnetoelastic sensor (ME) platform are gaining attention in chemical and biological sensing. ME sensors are constructed with amorphous ferromagnetic ribbons or wires which are analogous and complementary to piezoelectric acoustic wave sensors. ME sensors are excited with magnetic AC fields and in turn, they generate magnetic fluxes that can be detected with a sensing coil from a distance and hence these sensors are highly attractive for wireless biosensing. They have high tensile strength and are much more cost effective which makes them appealing for biosensor platform. The fundamental operating principle of the magnetoelastic sensors involves a change in sensor resonance frequency due to mass loading of the sensor. In biosensing, the change in mass is associated with the binding of a target analyte to a bioreceptor immobilized on the surface of the ribbon-like ME sensor. The ME sensor can be coated with various probe molecules to target analytes. A change in resonant frequency can be observed if an analyte binds to the magnetoelastic sensor which can be measured rapidly and accurately.

Ruan et al. (2004) have used ME sensors to detect staphylococcal enterotoxin Type B (SEB). The sensing configuration involves formation of a sandwich antigen–antibody complex by the primary affinity-purified anti-SEB antibody covalently immobilized on the surface of a mass-sensitive magnetoelastic sensor, the target SEB and the biotin-labeled secondary anti-SEB antibody. The study examined the response of the sensor by linearly varying the concentrations of SEB in the range of 0.5–5 ng/mL and demonstrated the sensitivity of magnetoelastic sensors. The detection limit was 0.5 ng/mL for a 1 h incubation period. Also the paper reported that, the cost of the sensor was approximately \$0.001/sensor, therefore it can be easily utilized as a disposable sensor.

Ong et al. (2006) reported the fabrication and application of wireless, remote-query ME sensors for the quantification of multiple biological agents. A six-sensor array was fabricated for the simultaneous measurement of *E. coli* O157:H7, staphylococcal enterotoxin B, and ricin by immobilizing anti-bacterial or anti-biotin antibodies onto a gold-coated ME sensor through self-assembled monolayer modification cross-linking the antibody with a bifunctional binding agent. The paper reported that the telemetry of magnetoelastic sensor information is by magnetic flux; hence, no direct connections are needed between the sensor and monitoring electronic equipment making possible a variety of *in situ* and *in vivo* monitoring applications. Recently, Huang et al. (2008) and Xie et al. (2009) developed a ME biosensor by immobilizing bacteriophage as bio-recognition element for the real-time *in vitro* detection of *B. anthracis* spores.

6. Electronic nose sensor

The development of an 'electronic nose' for pathogen detection has received considerable attention in recent years. Balasubramanian et al. (2005) used a commercially available Cyranose-320™ electronic nose system to identify *S. typhimurium* in inoculated beef samples. An

electronic nose containing an array of 32 conducting polymer sensors was used to obtain the odour patterns of the headspace of the meat samples. The volatile organic compounds emanating from vacuum-packaged beef strip was analysed. Their results proved that the electronic nose system was able to identify meat samples contaminated with *S. typhimurium* at a population concentration level $\geq 0.7 \log_{10}$ CFU/g.

Conducting polymers has been used as detectors in electronic nose systems. When gas is adsorbed by the sensor, conducting organic polymer sensors exhibit a change in resistance, which is sensed and delivered as the output (Arshak et al., 2009). Conducting polymer based e-nose sensor for foodborne pathogen detection has been reported by many researchers. Magan et al. (2001) used Bloodhound™ BH114, an electronic nose unit including 14 conducting polymer sensors, to detect the volatile profiles produced by uninoculated skimmed milk media or that inoculated with bacteria (*Pseudomonas aureofaciens*, *P. fluorescens*, and *B. cereus*) or yeasts. It has been reported that using discriminant function analyses it was possible to separate unspoiled milk and that containing spoilage bacteria or yeasts. The sensor array used was a useful discriminator of microbial volatile profiles. The paper discussed the potential for using an electronic nose system for the early detection of microbial spoilage of milk-based products.

Muhammad-Tahir and Alocilja (2003b) fabricated a disposable biosensor based on an electrochemical sandwich immunoassay using polyaniline for detection of foodborne pathogen, *E. coli* O157:H7. Their results showed that the biosensor was able to detect approximately 7.8×10^1 CFU/mL of *E. coli* O157:H7 in 10 min. Minett et al. (2003) coupled conducting polymers and the electron mediators for the detection of *L. monocytogenes* and the sensor was capable to detect *L. monocytogenes* at levels of 10^5 cells mL⁻¹ in 30 min. Also biosensors based on electronic conducting polymers were reported for the detection of fish freshness (Ghosh et al., 1998).

Recently, Arshak et al. (2007) reported the use of an array of conducting polymer composite sensors containing both carbon black and polyaniline to detect and identify the foodborne bacterial pathogens such as, *Salmonella* spp., *B. cereus* and *V. parahaemolyticus* through production of an individual response pattern for each bacterium. Their work demonstrates the potential application for the on site identification of food-borne pathogens where these sensors could be interfaced with handheld devices to quantify emissions emanating from samples of contaminated foods.

7. Summary and outlook

Though conventional pathogen detection methods are sensitive, they lag behind the analytical methods by detection time. However, the analytical techniques like optical and electrochemical detection have some disadvantages as well, considering sensitivity and cost. Therefore, new rapid methods are considered necessary for better performance. Optical techniques possibly provide better sensitivity relative to electrochemical detection, but they are expensive and complicated. In contrast, electrochemical techniques involve much simple procedures but for the detection of pathogens, it requires enhanced performance.

Though, numerous research efforts have been made during the past decades and in recent years for foodborne pathogen detection, in spite of everything it needs further improvement. Since foodborne pathogens are mostly present in very low numbers (<100 CFU/g) in the middle of millions of other bacteria, it is very difficult to detect them. So there are more chances for these microorganisms that may get lost during detection. Therefore a detection technique which is reliable, rapid, accurate, simple, sensitive, selective and cost effective has to be developed. In addition, it should be able to detect pathogens in very low concentrations of the samples and must be suitable for *in situ* real-time monitoring as well. Such a technique of detecting

pathogens would offer a great commercial advantage to food processing and food manufacturing sector. By using a biosensor which is rapid in detecting microbial pathogens, the uncontaminated foodstuffs can be released within hours or even minutes, rather than several days, which further prevent the necessity for refrigerated storage.

Acknowledgement

This research work is funded by Science Foundation Ireland (SFI) Research Frontiers Programme, ID no: 07RPF-ENEF500.

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