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Chapter 4

# Biosensors: A Fast-Growing Technology for Pathogen Detection in Agriculture and Food Sector

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**Abstract**

Agriculture and food have a greater role to play in order to achieve sustainable development goals. Therefore, there is a need to put an end to the effect of pathogens on food quality and safety. Pathogens have been recognized as one of the major factors causing a reduction in profitable food production. The conventional methods of detecting pathogens are time-consuming and expensive for the farmers in rural areas. In view of this, this chapter reviews the biosensors that have been developed for the detection of biological hazards in food and agricultural sectors. This chapter also lays emphasis on the impact of nanotechnology on building a fast, reliable, more sensitive, accessible, user-friendly and easily adaptable technology for illiterate farmers in the rural communities. On the whole, we have addressed the past and most recent biosensors that could ensure the quick delivery of vision 2030 which aims to end hunger and poverty.

**Keywords:** agriculture, food safety, pathogen, biosensor, nanotechnology

## 1. Introduction

Biosensor could be defined as an analytical device that produces a quantifiable signal proportional to the concentration of an analyte (i.e., pathogen or its cellular component or toxin molecule). The device comprises a transducer and biologically active elements or materials such as nucleic acids, enzyme, and an antibody that allows detection of an analyte by specific interactions [1]. Biosensors symbolize the end product of a quickly growing field, integrating fundamental and engineering and computer sciences to meet the urgent demands in various areas where its application is required [2–4]. There are different types of biosensors: acoustic, amperometric,

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electrochemical, optoelectric, calorimetric, potentiometric, immuno and piezoelectric. In this chapter, we report the earlier and recent trends in the usage of biosensors in the identification of pathogens that are responsible for biological hazards in food and agricultural sectors.

## 2. Traditional methods for pathogen detection in food and agricultural sectors

### 2.1. Polymerase chain reaction

The discovery and the development of polymerase chain reaction (PCR) have been a boon in the identification and characterization of pathogens [5–7] . PCR employs the following steps: isolation and purification of genomic DNA from plants or food-based pathogens, amplification of the target sequences followed by application of agarose gel electrophoresis for resolving the amplified products, and approximation of their fragment size by comparing with a standard DNA molecular mass marker [8].

The PCR is a nucleic-acid-based detection method. It is preferable than the other culture dependent techniques in the determination of microbial pathogens. The reasons being rapidity, accuracy, specificity, sensitivity, and the ability to identify small quantities of target nucleic acid in a given sample. It can also detect different pathogens in a single multiplex reaction. In addition, the detection of pathogens is not limited to the laboratory alone. Some portable PCR machines have been made available. The Smart Cycler is an example of portable PCR. It was developed to perform PCR for field identification of *Phytophthora ramorum* [9, 10]. Another example is the detection of *Sharka* virus in crude plant extracts of stone fruit trees, such as apricot, peach, and plum [11]. The International Plant Protection Convention has adapted this technique for the early detection of this devastating and destructive virus [12–14]. RT-PCR-based method has also been utilized to manage the emergence or presence of *Citrus tristeza* virus (a harmful virus causing tristeza syndrome in citrus) without any necessity for preparation of plant extracts or purifying nucleic acids [14–16]. This technique allows large-scale diagnoses thereby reducing the time and cost of analyses [12, 14].

Random amplified polymorphic DNA (RAPD) assays have been carried out on different isolates of *Fusarium poae* so as to discover the strain responsible for the head blight disease [17]. This method enabled them to identify markers common to all isolates. Turner et al. also performed RAPD profiling to screen and differentiate two different isolates of *Fusarium tricinctum* [18]. In another discovery, Schilling et al. utilized polymerase chain reaction to amplify, sequence and identify fungal pathogens *F. culmorum*, *F. graminearum*, and *F. avenaceum* [19]. Fraaije et al. invented a multiplex PCR assay that can sense and quantify pathogenic fungi, *S. tritici* causing leaf blotch; and *S. nodorum* causing leaf and glume blot, in wheat [20]. A TaqMan real-time PCR method has also been used to evaluate different species of *Fusarium* in wheat kernels [21, 22].

### 2.2. Culture and colony counting

The culture methods of identifying pathogens from food and agricultural based products involve the morphological and biochemical identification by staining and studying the

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metabolic profile of the pathogens. These methods require determination of the most suitable media that would favor their growth at different conditions. This may involve pre-enrichment, selective enrichment, biochemical screening, and serological confirmation. The major problems associated with using cultures for identifying pathogens are the high cost of media and the laborious and time-consuming techniques. In addition, they are not feasible for on the spot and real-time or rapid sensoring/identification of threat agents [23].

### 2.3. Immunology-based method

The immunological approaches for the detection of pathogens work on the principle of specific affinity between microbial antigens and monoclonal or polyclonal antibodies. They are used for rapid detection and identification of pathogens, including bacteria, viruses, fungus as well as their toxins. This method is very sensitive, rapid, selective and cost-effective. Latex agglutination and enzyme-linked immunosorbent assay (ELISA) are the techniques majorly used in food industry for identification of food pathogens like *Listeria monocytogenes*, *Salmonella*, *Campylobacter*, *Escherichia coli* O157: H7, *Listeria* and *Shigella*, *Staphylococcus aureus* [24].

### 2.4. Hand-held immunochromatographic assays (HHIA)

The hand-held immunochromatographic assays (test strips) are normally used for tentative or preliminary identification, both on-site and in laboratories. The test strips consist of nitrocellulose membrane immobilized with specific antibodies followed by a second antibody that is coupled to the colored particle. The liquid sample containing the analyte is then allowed to mix with the antibody-coupled colored particle. The analyte binds to the antibody-coupled particle and this complex migrate by capillary action along the nitrocellulose strip until it meets the immobilized antibody. The interaction produces a visible colored line indicating a positive result and vice versa. This type of assay takes only about 15 min to perform and the result can be read visually without any instruments. Therefore this detection technique is especially suitable for on-site identification. However, HHIA have two major limitations; limitation in the number of biological hazards that can be detected per strip and display of varying sensitivity levels with their respective target agents [25].

## 3. Biosensors used for pathogen detection in food and agricultural sector

### 3.1. Detection of food pathogens

Liébana et al. have developed a quick and simple biosensor based on electrochemical magnet immunosensing with *magnetic graphite-epoxy composite* (m-GEC) electrodes for the recognition of *Salmonella* in milk. The graphite-epoxy composite maintains a unique hybridization property that allows immediate immobilization of the DNA of the pathogens. This technique has a greater advantage over the cultural and biochemical/serological methods of detecting pathogens, as they do not require reagents and offers quick detection [26–30]. Based on this principle, Pividori and Alegret have also invented a biosensor that can detect the presence of

b-lactamase resistance in *Staphylococcus aureus* [31]. Oliveira Marques et al. invented a gold nanoparticle-based biosensor with graphite-epoxy composite electrodes for the identification of *Salmonella* IS200 [32]. A double-tagged PCR strategy had been used for the detection of pathogenic bacteria, enterohemorrhagic *E. coli* O157: H7. The biosensor works on electrochemical magnet genosensing and allows electrochemical real-time quantification of an amplicon [33]. Ricci et al. have developed an electrochemical biosensor that can detect pathogens such as *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* in milk [34].

Majumdar et al. developed an amperometric biosensor which was able to detect *Staphylococcus aureus* in food samples such as milk, cheese, and meat [35]. Banada et al. utilized light scattering sensors for the detection of microorganisms in vegetable and meat samples [36]. Shriver-Lake et al. also used an optical (fluorescence)-based portable Naval Research Laboratory (NRL) array biosensor that can sense the presence of *Salmonella typhimurium* in milk and apple juice within 45 min [37]. Karsunke et al. invented a multiplexing optical (luminescence) biosensor which can sense the presence of *E. coli* O157: H7, *S. typhimurium* and *Legionella pneumophila* in any sample in a disposable microarray format. In their discovery, immunospecific antibodies were immobilized in a microarray format [38]. Several authors have described many multiplexing biosensors that make use of polymerase chain reaction. Koets et al. in their study developed the use of magnetoresistance biosensor that can sense the presence of *E. coli* and four different antibiotic-resistant genes in *Salmonella* spp. along with a double-tagged PCR amplification step [39]. Bai et al. used a biosensor that has a microarray approach with biospecific DNA probes immobilized on a sensor surface for the sensing of 11 food-borne pathogens present in beef and pork meat [40]. Schütz et al. developed a biosensor that can detect the volatile compounds emitted by the pathogenic fungus *Phytophthora infestans* that is responsible for spoilage in potatoes [41].

### 3.2. Detection of animal, poultry, and dairy pathogens

Ellis et al. were able to develop a sensor that could detect breath-derived 500 volatile organic compounds. The analysis helped in identifying Bovine tuberculosis (*M. bovis*) in affected cattles [42]. Tarasov et al. developed a direct potentiometric biosensor that could detect Bovine Herpes Virus-1 viral protein. The biosensor is sensitive and selective to anti-IgE present in commercially available anti-Bovine Herpes Virus-1 antiserum as well as in real serum samples from cattle. The biosensor can also be easily used with *point-of-care* devices and ELISA [43]. ELISA and PCR-based methods have been utilized for quick detection of bovine viral diarrhea virus, especially for the onsite monitoring and early diagnosis of the bovine viral diarrhea virus infection in animals [44, 45]. In addition, Luo et al. have established an electrospun biosensor which works on the principle of capillary separation and conductometric immunoassay for the early sensing of bovine viral antibodies where the sensing time takes 8 minutes [46]. Microparticle immunoagglutination assay on a microfluidic chip using forward light scattering measurements have also been developed to sense the presence of bovine virus particles [47]. A new biosensor with a miniaturized gold electrode which works on impedance spectroscopy that can detect the presence of H7N1 has also been developed [48, 49]. Xu et al. have developed an interferometric biosensor immunoassay which can sense different avian influenza strains, especially H7 and H8 [50]. Bai et al. also developed a simple and portable biosensor with DNA aptamers as recognition elements in portable surface plasmon resonance (SPR) which can sense

the presence of H5N1 available in poultry swab samples [51]. Ye et al. have also developed a biosensor that is based on the principle of Luminescence 645 resonance energy transfer for the quick detection of H7 strain [52], while Guo et al. developed a biosensor which consists of an indium-tin-oxide thin-film transistors built on a glass substrate for immune detection of H5N1 antibodies [53]. Lum et al. developed a nano-based biosensor that works on the principles of immune magnetic nanoparticles for the detection of H5 subtype virus [54].

Neitzel et al. have developed a biosensor that can detect the presence of mastitis in any milk product [55]. Duarte et al. had also developed a biosensor that couples immune assay with magnetic nanoparticles [56]. Fűtő et al. developed selective amperometric methods that could sense the presence of spoilt and affected milk [57]. The spore-based biosensor is another novel strategy that has been developed to detect the presence of contaminants, including aflatoxins, antibiotics and microbial pathogens in milk. Balhara et al. developed a biosensor that can detect the presence of *L. monocytogenes* and *Listeria* spp. in milk products. This sensor employs the enzyme-substrate reaction that produces a color change and can be easily visualized [58]. Kumar et al. had also developed a biosensor that utilized two-stage enzyme assay for the detection of *Enterococci* spp. in milk [59].

### 3.3. Detection of pathogens in plants

A high-density microelectrode array biosensor was developed by Radke and Alocilja [60]. The biosensor can detect *E. coli* O157: H7 bacteria in food materials. They discovered that change in impedance of the biosensor is directly proportional to the number of bacteria on the biosensor surface. They detected up to 10 cells of *E. coli* O157: H7 by testing the biosensor in different concentrations of bacteria in lettuce. The advantage of this sensor is that it is field-deployable, easy to use, portable, and reagent-less and provides result in minutes compared to hours or days in conventional methods. Kim and Park developed a flow-type antibody sensor using quartz crystal microbalance chip as biological component and transducer to detect *E. coli* in drinking water, beef, pork, and dumpling. The developed sensor measures frequency changes due to mass deposits which are produced by antigen-antibody interaction [61]. Mendes et al. developed a biosensor that can detect the pathogenic fungus *Phakopsora pachyrhizi* that had been reported to cause Soybean rust [62]. Papadakis et al. also had developed an acoustic-based biosensor (the Quartz Crystal Microbalance) that could sense three out of the most reported plant pathogens, i.e., *Ralstonia solanacearum*, *Pseudomonas syringae pv tomato* and *Xanthomonas campestris pv. Vesicatoria* [63].

### 3.4. Detection of mycotoxins

Carlson et al. developed a fluorometric biosensor to detect and quantify aflatoxins. These toxins are produced by a family of fungi and are commonly found in a variety of agricultural products. The device developed by Carlson et al. operates on the principle of immunoaffinity for specificity and fluorescence for a quantitative assay [64]. Pohanka et al. and Ben Rejeb et al. used Electrochemical (amperometric) antibody-based biosensor to detect the presence of Aflatoxin B1 in spices and olive oil respectively [65, 66]. Wang et al. used an electrochemical (amperometric) antibody/enzyme biosensor to detect Aflatoxin M1 in milk [67]. Asuncion

Alonso-Lomillo et al. used an electrochemiluminescent aptamer biosensor to detect the presence of Ochratoxin A in beer and coffee samples [68]. Panini et al. used electrochemical (amperometric) antibody biosensor to detect the presence of zearalenone in corn silage [69]. The presence of deoxynivalenol, T-2, and HT-2 toxins was also detected in cereals and baby food with the help of optical (SPR) antibody biosensor [70, 71].

## 4. Application of nanotechnology-based sensors in agriculture and food sectors

### 4.1. Nanomaterial-based sensors for food industry

The food industry as mentioned earlier is continuously challenged by the occurrences of foodborne diseases. WHO in its report for the year (2015) estimated 420,000 deaths occurring every year due to consumption of contaminated food, of which 125,000 deaths are of children under the age of 5, bearing a 40% burden of foodborne diseases [72]. Foodborne disease can be defined as "any disease usually either infectious or toxic in nature, caused by agents that enter the body through ingestion of food." The causal agents are bacteria, viruses, and protozoa, fungal or bacterial toxins, metal ions, and pesticides. Some of the important pathogenic organisms categorized are *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli*, *Brucella*, *Listeria monocytogenes*, *Salmonella typhi* and *paratyphi*, *Shigella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus* [73]. In spite of the advances in healthcare, food-borne diseases are likely to remain a global phenomenon even in the next decade. The contributing factors are urbanization and changes in consumer habits, increased demand for food varieties resulting in a global food cuisine trade, changes in agricultural practices and food processing methods and climate change. The WHO has thus placed food safety as one of its top 11 priorities [74]. In order to manage and contain foodborne diseases, it is important to develop low cost ready to use tests for immediate detection of pathogenic contamination or presence of toxins that would replace the conventional methods. Some of the conventional methods that are routinely used are PCR based methods and immunoassay-based techniques.

These methods are robust and sensitive as they allow the detection of pathogens by targeting specific nucleic acids or proteins. However, the requirement of an expensive instrument and chemical reagents, experienced personnel, large sample preparation and slow generation time prevent the immediate detection of pathogens thus delaying preventive treatment in patients [75, 76]. Thus, the shift has been to the development of easy to use, rapid and sensitive on the site detection and also stable and portable detecting kits. Nanotechnology has paved way for such developments in the last decade. The versatility of nanomaterials has made possible the development of sensors in the food industry for monitoring the environment and food quality [77]. Some of the advancements in the design and development of nanoparticle-based sensors for food safety are discussed below.

#### *4.1.1. Gold nanoparticle (AuNP)-based sensors*

*E. coli* O157: H7 is the serotype among *E. coli* strains associated with foodborne diseases. A circulating flow piezoelectric biosensor (PEB) was developed to detect *E. coli* O157: H7. The

PEB has *E. coli* O157: H7 *eaeA* gene specific AuNP-conjugated thiolated probe that acts as mass enhancer and sequence verifier. The detection limit obtained in PEB is $1.2 \times 10^2$ CFU/mL in the linear working range of $10^2$–$10^6$ CFU/mL [78]. The AuNP conjugated with *E. coli* O157: H7 antibodies were also developed for detecting *E. coli* O157: H7 in milk. Screen printed carbon electrodes (SPCE) having 13 nm AuNP were fabricated with *E. coli* O157: H7 specific antibodies conjugated with horseradish. Hydrogen peroxide and ferrocene dicarboxylic acid (FeDC) were used as substrates. AuNP and FeDC enhance the detection limit to $10^2$–$10^7$ CFU/mL [79]. The AuNP based electrochemical immunoassay was also developed for detecting *S. typhimurium* [80]. Polystyrene immobilized with *S. typhimurium* specific monoclonal antibodies that were further layered with AuNP-conjugated polyclonal antibodies were used as the probe. In the presence of copper enhancer solution and ascorbic acid, the bacteria bind to the AuNP-conjugated polyclonal antibodies. The copper released upon reduction, bind to the AuNP thus allowing direct detection of *S. typhimurium* by anodic stripping voltammetry. The detection limit for this AuNP based immunoassay is 98.9 CFU/mL. Colorimetric based AuNP-conjugated with anti-Salmonella antibody has also been developed for detecting and selectively targeting *S. typhimurium* [81]. The AuNP based sensors were also developed for detecting mycotoxins in food products. AuNP-aptasensor for detecting aflatoxinB1 was developed by Hosseini et al. [82]. The presence of aflatoxin destabilizes the AuNP-aptamer and causes aggregation of AuNP. The color change from yellow to purple allows the detection of the presence of the toxins. Similar AuNP-aptasensor for detecting Aflatoxin B2 was developed by Luan et al. The detection here was also based on colorimetric method [83].

#### *4.1.2. Magnetic nanoparticle (MNP)-based sensors*

Magnetic nanoparticle-derived sensors are one of the widely used sensors for detecting and removing food contaminants. The large surface area of MNPs makes them one of the best supports for immobilization of functionalized surface groups thereby improving the loading control and immobilization efficiency [84]. D-mannose functionalized MNPs were used for detecting *E. coli* cells at $10^4$ cells/mL. These modified MNPs when incubated with fluorescently labeled concanavalin allowed the magnetic separation and visualization of the cells [85]. Antibody conjugated MNPs were developed to detect *Salmonella* in milk. The immobilized antibodies allowed the capturing of the bacteria that are further separated by application of magnetic field. The separated cells are then exposed to antibody immobilized $TiO_2$ nanocrystals. Thus, the antibody-MNP-$TiO_2$ nanocrystals are magnetically separated and the unbound $TiO_2$ nanocrystals are determined using the UV-visible spectrophotometer. A detection limit of 100 CFU/mL was obtained from milk samples [86]. Amine functionalized MNPs were also developed for rapid detection and capturing of both gram-positive and gram-negative bacteria from water and food matrices. Organisms that showed high adsorption affinity are *Sarcina lutea*, *S. aureus*, *E. coli*, *B. cereus*, *B. subtilis*, *Salmonella*, *P. vulgaris*, and *P. aeruginosa.* It was shown that the amount of amine functionalized MNPs and the ionic strength of the buffer was crucial for mediating fast and effective interaction [87].

#### *4.1.3. Quantum dots (QD)-based sensors*

Semiconductor QDs show size-dependent optical and electronic properties making them most suitable for fluorometric-based sensors [88]. The most commonly used are the CdSe quantum

dots [89]. The QD-derived fluorescent biosensor was developed for detecting *S. typhimurium* in chicken carcass wash water. Magnetic beads coated with anti-Salmonella antibody was used for capturing the bacteria that was further made to react with a biotin-labeled anti-Salmonella antibody. This facilitated the reaction of biotin to the streptavidin-coated QDs. The fluorescence intensity is a direct measure of the cell number in the sample. The detection limit obtained was about $10^3$ CFU/mL [90]. The CdSe QDs derived sensors were also developed for detection of *Cholera*, *Shiga* toxin and *Staphylococcal* enterotoxin A.

### 4.2. Nanomaterial-based biosensors for agriculture

The use of nanobiosensors has been regarded as the more advantageous approach for detecting pathogens in healthcare and food industry as mentioned above. Their rapid and high sensitivity further extends their application in agriculture for disease assessment. Fluorescent silica nanoparticles (FSNP) conjugated with antibodies were successfully used for detecting plant pathogens such as *Xanthomonas axonopodis pv. vesicatoria* which causes bacterial spot disease in tomatoes and peppers [91]. Copper oxide (CuO) nanoparticles have been used in the detection of the *A. niger* fungi [92]. In addition, silver-based nanoparticles, AgNPs are commonly used for detecting contaminants and microbial pathogens in the soil and water bodies. Thus the use of nanosensors has allowed plant disease forecasting and disease management in agriculture to an admissible level [93].

## 5. Recommendations and future trends

There is a need to develop biosensors that would be effective and reliable for the routine utilization especially in the area of food and agriculture. Therefore, there is a need to develop biosensor that has the following characteristics in one device: hand-held, and portable, viable cell countability, single button device, easy utilization, accurate strain and species determination, selectivity and short detection time. And most importantly, the biosensor must be inexpensive with simple configuration for access to the illiterate farmers in developing countries.

## 6. Conclusions

Because of the useful features of biosensors, their utilization in the bio-monitoring of biological hazards, commonly recorded in agriculture and food sectors has been necessitated. The constant application of pesticides in controlling pathogens has led not only to pathogen resistance but also, bioaccumulation and biomagnification of the chemicals with subsequent health hazards and environmental pollution. Therefore, the demand for biosensors in the market has increased tremendously. Biosensors should be within the reach of food handlers and agro-allied industries to enable them to monitor and determine the presence of pathogens in their food and agricultural products.

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## Conflict of interest

The authors declare no conflict of interest.

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