Pathogen detection, food-borne

The presence of microorganisms in food is a natural and unavoidable occurrence. Cooking generally destroys most harmful bacteria, but undercooked foods, processed ready-to-eat foods, and minimally processed foods can contain harmful bacteria that are serious health threats. Meat, dairy, and poultry products are important reservoirs for many of the food-borne pathogens, including Salmonella, Campylobacter, Listeria, and Escherichia coli O157:H7. Animal by-products, such as feed supplements, may also transmit pathogens to food animals (for example, Salmonella and bovine spongiform encephalopathy). Seafood is another potential source of food-borne pathogens, such as Vibrio, Listeria, and Hepatitis A. Infectious doses of many of these pathogens are very low (~10 bacterial cells), increasing the vulnerability of the elderly, infants, and people with immunological deficiencies or organ transplants.

Researchers are continuously searching for sensitive tools that are fast, accurate, and ultrasensitive. In recent years, there has been much research activity in the area of sensor development for detecting pathogenic microorganisms.

Conventional microbiological methods. Conventional culture methods remain the most reliable and accurate techniques for food-borne pathogen detection. Conventional methods include blending of the food product with a selective enrichment medium to increase the population of the target organism; plating onto selective or differential agar plates to isolate pure cultures; and examining the cultures by phenotypic analysis or metabolic fingerprinting (monitoring of carbon or nitrogen utilization). A major drawback is that these methods are labor-intensive and take 2–3 days for results, and up to 7–10 days for confirmation. To avoid delays, many of the modern detection tools use a conventional method along with an automated or semiautomated DNA, antibody, or biochemical-based method (Fig. 1). These methods allow detection in 3–4 days. See PATHOGEN DETECTION.

Antibody methods. The basic principle of antibody-based detection (immunoassay) is the binding of antibodies to a target antigen, followed by the detection of the antigen-antibody complex. Antibodies are produced by the body in response to a specific invading pathogen. Experimentally, these molecules are produced in laboratory animals against a specific antigenic component of the pathogen or toxin. The most important characteristic of an antibody is its ability to recognize only the target antigen in the presence of other organisms and interfering food components. In addition, the successful use of antibodies to detect pathogens depends on the stable expression of target antigens in a pathogen, which are often influenced by temperature, preservatives, acids, salts, or other chemicals found in foods.

In a direct immunoassay, antibodies are immobilized onto a solid support and then test samples are added. Once specific antigen-antibody binding takes place, the complex is detected. The most common detection method is to use another antibody (sandwich format of antigen between two antibodies) conjugated to an enzyme or a fluorescent dye specific for the antigen. When enzyme activity is quantified by using a substrate that produces a colored product, the assay is called an enzyme-linked immunosorbent assay, or ELISA (Fig. 2). A spectrofluorometer or an epifluorescence microscope is used to measure emission of fluorescence when a fluorescent-labeled antibody is used. The sensitivities of these methods are in the range of $10^7$–$10^9$ bacterial cells, and the assays take about 3–4 h to complete.

Another antibody-based method is the lateral flow device (Fig. 2). Bacterial cells or antigens are detected by a double-antibody sandwich format on a membrane. When a sample is introduced, it binds to antibody-gold conjugates. This antigen-antibody complex migrates on the absorbing membrane and binds to another antibody (antigen-specific) resulting in a visible band. This type of assay is very fast and simple, and results can be obtained in 10–15 min. The assay, however, requires a large number of cells ($10^7$–$10^9$ cells) and may lack specificity. Another key development in the antibody-based detection technology is the use of antibody-coated magnetic beads (Fig. 2), which are able to capture and concentrate bacterial cells from complex food matrices. These captured bacterial cells are then detected by various methods, including biosensors.

Biosensors. Biosensors are devices that detect biological or chemical complexes in the form of antigen-antibody, enzyme-substrate, or receptor-ligand. Most biosensors that have been developed for pathogens have been tested only with pure bacterial cultures. For such applications, the pathogens are first
separated from the food and then applied to the sensor. Very few studies have actually attempted to detect bacteria directly from food. Food is extremely complex material consisting of fats, proteins, carbohydrates, chemicals, and preservatives, as well as different acidities, salt concentrations, and colors. The application of nanotechnology (interrogating nanosize particles on sensors) to detect pathogens from complex food systems is an incredible challenge. Moreover, the populations of target microorganisms are often extremely small compared with the indigenous ones. Consequently, clever strategies need to be used to detect such low numbers of pathogens directly from food (Fig. 1).

**Fiber-optic biosensor.** The use of optical fiber is being investigated for the real-time detection of biological agents (such as bacterial cells, toxins, or spores) in the air, soil, or environment. The fiber-optic biosensor operates by covalently linking a specific antibody to the fiber-optic cable, binding a target antigen to the antibody, and then detecting the antigen-antibody complex by means of a secondary antibody conjugated to molecules that can be stimulated to emit fluorescent light which is measured by a laser detector. Antibody-coupled fiber-optic biosensors are being developed for the detection of botulinum toxin, staphylococcal enterotoxin, *E. coli* O157:H7, *Listeria*, and *Salmonella*.

**Surface plasmon resonance sensor.** A surface plasmon resonance sensor is an optical sensor that is capable of characterizing the binding event of biomolecules in real time (few seconds to minutes) without the need for labeling molecules, by detecting differences in the intensity of light reflecting off a sensing surface. Antibodies or receptors for detection of food-borne pathogens or toxins are immobilized in a biolayer attached to a sensing surface. When binding takes place, it alters the angle of light reflected off the medium, resulting in a signal. If the compound cannot be accommodated within the boundaries of the biolayer, it will not provide a strong signal. Although this system has been used for detection of whole cells of *E. coli* O157:H7, *Salmonella*, and *Listeria* at variable concentrations, it showed strong signals with small toxin molecules, such as staphylococcal or botulinum.

**Electrochemical immunosensors.** Electrochemical immunosensors are based on conventional antibody-based enzyme immunoassays. In these applications, catalysis of substrates by an enzyme conjugated to an antibody produces products (ions, pH change, or oxygen consumption) capable of generating an electrical signal on a transducer. Potentiometric, capacitive, and amperometric transducers have been used for such applications. In amperometric detection, alkaline phosphatase conjugated to an antibody...
hydrolyzes nitrophenyl phosphate to produce phenol, which could be detected by voltammetry. In light-addressable potentiometric sensors (LAPS), urease attached to the antibody hydrolyzes urea, resulting in the production of carbon dioxide and ammonia, causing a change in the pH of the solution. A silicon chip coated with a pH-sensitive insulator and an electrochemical circuit detects the alternating photocurrent from a light-emitting photodiode on a silicon chip. These sensors are very sensitive and have been used to detect Salmonella and E. coli O157:H7 in 30–90 min.

Microfluidic biochips. Biochips are microelectronic or microfabricated electronic devices (such as semiconductor chips) that are used for monitoring the activities of biomolecules. Biochips have been used for monitoring eukaryotic or prokaryotic cell growth and DNA hybridization. Microbial growth on a biochip is measured by an impedance analyzer on a microscale level. The microbial metabolism of sugars or other substrates produces acids and ionic species. If the electrical property of the medium is monitored over time, the conductance and the capacitance increases while the bulk impedance decreases. A concentration level of 50–100 cells per nanoliter can be detected on a chip. In its current setup, the specificity of this type of sensor depends on the specific growth medium used. Alternatively, a specific antibody can be used to capture a target bacterium on the chip.

Cell-based sensor. One key feature of pathogenic bacteria is that they interact with eukaryotic cells as part of the infectious process (Fig. 3). A gross change in the eukaryotic cell structure by a pathogen can be observed microscopically. However, if a sensor can be used to measure the change occurring at a single cell, the sensitivity of the detection is greatly enhanced. In principle, the compartments of a cell are separated from the surrounding medium by a cell membrane, which consists mainly of highly structured electrically insulating phospholipids. The electrical properties of the biological membrane can be modeled as a resistor and capacitor network. These membrane properties affect the conductivity of the cell system at alternating-current frequencies. Any external factors, such as live bacteria or active cytotoxins that affect the integrity of the membrane, alter the conductivity and provide a signal. The signal (impedance of the cells) can be measured with an interdigitated microsensor electrode. This type of sensor shows great promise for detecting potential pathogenic food-borne microorganisms and toxins.

Piezoelectric (PZ) biosensors. These sensors detect changes in the mass on the surface of a quartz crystal. Antibodies are used for specific binding of the analytes, which increase the mass of the crystal while the resonance frequency of the oscillation decreases proportionately. Salmonella and Listeria have been detected with this system. A variation of the piezoelectric system called quartz crystal microbalance consists of a thin quartz disc with implanted electrodes.

For background information see BIOELECTRONICS; BIOSENSOR; CLINICAL MICROBIOLOGY; FOOD ENGINEERING; FOOD MANUFACTURING; FOOD MICROBIOLOGY; TRANSDUCER in the McGraw-Hill Encyclopedia of Science & Technology.

Arun K. Bhunia; Amanda Lathrop


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