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Review

# Biosensors of bacterial cells

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ABSTRACT

Biosensors are devices which utilize both an electrical component (transducer) and a biological component to study an environment. They are typically used to examine biological structures, organisms and processes. The field of biosensors has now become so large and varied that the technology can often seem impenetrable. Yet the principles which underlie the technology are uncomplicated, even if the details of the mechanisms are elusive. In this review we confine our analysis to relatively current advancements in biosensors for the detection of whole bacterial cells. This includes biosensors which rely on an added labeled component and biosensors which do not have a labeled component and instead detect the binding event or bound structure on the transducer. Methods to concentrate the bacteria prior to biosensor analysis are also described. The variety of biosensor types and their actual and potential uses are described.

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## 1. Introduction

The detection and identification of bacterial pathogens remain a high priority goal. There are many applications, with detection of pathogens in clinical samples a task requiring especially rapid and accurate determinations. Rapid detection often means the difference between life and death for the patient. Detection of pathogens from environmental samples, such as water, food, and in air samples are also necessary to maintain public health. A rapid answer is always preferred because it allows faster decision-making on treatment or safety of the environment, while specificity is needed to inform appropriate clinical treatment or to accurately assess the danger to the public. The identification and quantification of bacteria has traditionally been performed using biochemical assays and growth on selective and differential agar media. These methodologies have worked well, however their procedures are time-consuming, often two to three days, and require skilled personnel to run the appropriate tests and interpret results. Current technology is at a point where biosensors can be used to detect pathogenic bacteria in a rapid and cost-effective manner. Yet technology alone will not solve problems if the cost is prohibitive.

A biosensor is a scientific instrument that incorporates a biomolecule in order to detect and possibly quantify an analyte. It will also have a transducer component, which is necessary to convert the biological event into a measureable signal. The signal is usually electrical in nature. The biomolecule is often a nucleic acid, an antibody or an enzyme, but the list of possible biomolecules is increasing as the number of sensing methods has increased. Lectins, cell surface receptors, and complex polysaccharides can be used to bind to biological targets. The field is now so large that specialized journals are devoted to the topic. A microbiology researcher will probably understand the biological component perfectly well, yet may have trouble understanding the transducer component. Throughout this review particular attention is given to the transducer technology.

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After isolation of bacteria, identification is now routinely performed using 16S rRNA sequencing. There are many biosensor methods that incorporate the rRNA sequence, and it would be appropriate to include nucleic acid-based biosensors in this review. However, except for a brief comparison of these valuable methods this review will be limited to biosensors of whole bacterial cells. Identification of whole bacterial cells is a good alternative to nucleic acid methods since they avoid the step of nucleic acid isolation and purification. There is also the issue of live versus dead bacteria in any sample. 16S rRNA collection does not discriminate between live and dead cells; plating on agar media will identify only living bacteria while missing the viable-but-not-culturable (VBNC) cells. VBNC cells are still important because they have the potential to recover and multiply. Relatively little is known about the VBNC state, although conditions for regrowth have been described (Coutard et al., 2007) and a resuscitation factor has been described (Ayrapetyan et al., 2014). Therefore they should be considered as potentially pathogenic (Besnard et al., 2002). A DNA hybridization method would identify the bacteria but not distinguish live and dead cells, while an RNA hybridization might suffice if the RNA species were carefully chosen. In dead cells the RNA degrades quickly.

Biosensors of whole bacterial cells may also detect dead cells, but typically depend on the structural integrity of cell surface biomolecules which may also degrade quickly after the cell dies. Ramamurthy et al. (2014) describe some of the targets which can be used to detect the VBNC cells. Even with the limitation to biosensors of whole bacterial cells this review should not be considered a comprehensive work. The field is vast and new ideas occur constantly. From the examples shown here much can be discovered about the latest methods of detecting biomolecules in general, and may stimulate additional innovative ideas.

## 2. Concentration and sequestration

A critical facet of cell detection is the isolation of the cells from the bulk phase. The bacteria of interest are a tiny fraction of the volume and are probably only a small fraction of the total bacterial population present in the sample. The chances of the bacterial cells contacting the transducer of the biosensor are thus very small if only a small volume is examined. Effectively, the same number of target cells must be present in a smaller volume to allow contact with the biosensor. This is also a major problem with a real-time, on-line biosensor, since it is unlikely that the sample could ever be concentrated in a high-volume flowing environment.

Reducing the volume might be accomplished through centrifugation or filtration of a sample. Resuspension of the collected particles in a smaller volume results in a higher concentration. However, there are alternatives that allow efficient collection of bacteria without the need for physical handling of the bulk fluid. The use of magnetic nanobeads has proven to be a popular and resourceful way to capture and concentrate the target bacterial cells. The recent review by Bohara and Pawar (2015) gives an extensive overview of the uses of magnetic nanoparticles. Conceptually, the simplest application is when magnetic particles are modified with an antibody and are mixed into a sample that may contain the bacteria of interest. The antibodies attach to the bacteria and the nanoparticles are then removed from the bulk phase using a magnet. A wash buffer is typically applied to remove any loosely bound material. This is the basis of immunoseparation (Wang et al., 2016).

The antibody sandwich assay, described in detail below, can take advantage of immunoseparation if a second antibody is used that has a detectable label. A good example is the use of magnetic nanoparticles to facilitate the discovery of *E. coli* O104:H4, an enterohemorrhagic strain (Luciani et al., 2016). It is often the case that enterohemorrhagic bacterial strains are found in milk which has not been effectively pasteurized or in ground beef which has not been sufficiently cooked. In this application the target was actually the lipopolysaccharide of the cell, defined by the O104 antigen. Here two monoclonal antibodies were used: one was attached to magnetic nanobeads and used to capture the antigen in a milk sample. This demonstrates that the antibodies need not be attached to a fixed surface and may be suspended in a liquid sample where exposure to the bacteria might be increased. After concentration the bacteria were enumerated using a simple selective media and colony counting. They did not take the idea to the next logical step and create a rapid assay.

The core of the magnetic nanoparticle must be a ferromagnetic element, such as iron, nickel, or cobalt. Iron compounds are very commonly used for this purpose. Colloidal suspensions are typically produced, with particle diameters in the range of 5–200 nm. A surface coating is recommended since iron will oxidize quickly. Ideally, the surface coating should protect the iron core from oxidation while increasing the particle dispersion in water. This last point is critical since clumping of particles will be counterproductive. If possible, the surface coating might be used to attach the next particle modification. For example, a coating that also allows easy attachment of antibodies is very useful. Some coatings are deposited as thin films such as gold or graphite, while other coatings are common polymers like polyethylene glycol (PEG), polyethylene imine (PEI) and polyvinyl alcohol (PVA). There are many surface coating applications which have been described, too many to adequately address here.

A common mechanism of detection is the attachment of the target bacteria using a biomolecule, after which they are concentrated near or at the transducer. In theory, any molecule that bacteria of interest are known to bind to can potentially be used as the biomolecule in a biosensor. Specificity of the interaction is obviously important as is the affinity of the target for the probe biomolecule. One of the more interesting applications involves the attachment of the antibiotic vancomycin to magnetic nanoparticles (Chung et al., 2011). Vancomycin binds to the small peptide in the peptidoglycan cell wall of actively growing cells. When vancomycin is attached to the nanoparticle it is able to bind to many Gram positive genera of bacteria in an environmental sample. Sequestration by magnet then concentrates the bacteria. The method selects for bacteria which have a certain marker, and if more than one genus or species has this marker they all have an opportunity to be detected.

Any biomolecule which recognizes or attaches to a bacterial cell can potentially be used in a biosensor. This includes specific recognition molecules on eukaryotic cell surfaces, which are often the targets for bacterial attachment prior to infection. Lectins, which bind carbohydrates, should work as well to bind bacterial surface structures. An obvious choice for a biomolecule is an antibody, the use of which will be described below. They combine specificity and sensitivity and so are ideal for identifying small quantities of specific markers. Production of antibodies, either monoclonal or polyclonal, is an advanced technology. Antibodies, usually IgG, are available commercially for many targets while businesses offer services to produce antibodies against targets on demand. There are established techniques to attach antibodies to a variety of surfaces such as plastic and glass (Shriver-Lake et al., 1997). As mentioned above, they can easily be attached to a magnetic nanoparticle and used to isolate the bacteria from a sample.

In practically any biosensor there is a possibility for non-specific binding of organic compounds. This is always an aspect that must be addressed in assembling a new method or investigating a new sample source. The issue of non-specific binding during an antibody-based assay is conceptually easy to understand. Many immune-based assays are based on two antibodies which both recognize an antigen, the so-called sandwich assay. The first antibody must be attached to a surface, whether it is an immobile surface as part of the entire biosensor or a nanoparticle that must be concentrated. The antibody captures the target antigen, such as a bacterial cell or cell component. Then the second antibody, usually bearing a convenient marker (e.g. fluorescent molecule, enzyme) attaches to the target as well, which will produce the signal of the biosensor. This describes a label-based biosensor. If the technique detects the actual event of antibody:antigen binding it

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