Chapter

Applications of Phage-Based Biosensors in the Diagnosis of Infectious Diseases, Food Safety, and Environmental Monitoring

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Abstract

Bacteriophages are interesting entities that parasite bacteria. After infection, they gain new properties such as selectively binding proteins, thanks to genetic manipulation capability. Owing to this, they may be applied as recognition elements in different types of biosensors. Combining bacteriophages with various transducers can then result in the construction of innovative sensor designs that could improve the quality of food safety and environmental monitoring services. Contamination of foods by bacterial pathogens, such as Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, and Salmonella typhimurium, results in human infection that can severely affect the immunocompromised, the elderly, and pregnant women. Therefore, this chapter discusses the use of bacteriophages, or their derived peptides, as new sensing elements for the recognition of biomarkers, and the development of highly effective diagnostics tools for early prevention of food-borne infections.

Keywords: biosensors, phages, Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium, E. coli 0157:H7

1. Introduction

Countless pathogenic bacteria are causing different illnesses in humans and animals, resulting in several outbreaks of diseases worldwide. Every year, millions of individuals get infected by these bacteria, while the frequent sources of infections are health care–associated, food- borne, and/or waterborne [1]. The Centers for Disease Control and Prevention (CDC) assesses around 1.7 million clinical infections, and more than 34 million cases due to foodborne and waterborne pathogens infections, per year [2]. For instance, the prevalence of food poisoning has become a serious health issue worldwide due to climate change. The most common symptoms include diarrhea, nausea, vomiting and stomach cramps. However, food poisoning is dangerous for children and the elderly, as well as patients with weakened immune systems or chronic health conditions [3]. Early detection of these bacterial contaminants is essential in fighting against the rising infections and would help in offering best suitable intervening therapies for early prevention. There are various conventional methods for the detection of water-borne
and foodborne pathogens, which include specific biochemical and microbiological tests [4]. However, these methods are time-consuming and often require long pre-enrichment steps of the microorganisms and then culturing them on selective media. Another major problem, viable bacterial strains can become non-cultivable in the environment (VBNC), which leads to a failure to isolate a pathogen from a contaminated sample, as well as an underestimation of pathogen numbers. Consecutively, mass spectrometry has been recommended to enhance the speed and sensitivity of culture methods, but this approach is expensive and necessitate expertise for analysis and interpretation of the data. In contrast, rapid and simple biochemical immunoassays such as enzyme-linked immunosorbent assay (ELISA), can have low sensitivity for the detection of bacterial pathogens [5]. As a solution to the challenging issue, the development of biosensors, particularly phage-based, for bacterial detection in food, environmental, and healthcare associated samples has accelerated since the last few decades (Figure 1). In the past, bacteriophages have been used for diagnostic purposes, but to a limited subset of settings due to lack of engineering methods. Most recently, thanks to DNA sequencing technologies and more sensitive reporter systems such as luciferase, bacteriophages are engineered to express reporter proteins that aid in identifying a particular type of bacterial cell, which is susceptible to infection by this specific strain of bacteriophage [6]. This in turn have expanded the scope of innovative biosensor diagnostics. Thus, phages are established, unique bio-probes, due to their specificity, selectivity, and enduring tough environmental conditions. For example, foodborne and waterborne pathogenic bacterium, *Salmonella typhi* has been successfully detected under the VBNC state using a lytic phage biosensor [7]. Furthermore, bio-probes from phages such as antibodies, proteins, DNA/RNA aptamers, and carbohydrates have been used in transducer development for various analytical approaches to offer specific detection. These include bioluminescence, electrochemical, fluorescence, magnetoelastic, nanoparticle-based, surface plasmon resonance, etc. Other alternatives of bio-probe would involve phage receptor binding proteins (RBPs) and phage-display peptides (PDPs), which has been successfully applied to detect bacterial pathogens in food samples e.g., milk, chicken ... [8]. In the following context, enhancing the transducer surface of biosensor would increase selectivity, sensitivity, and consistency. This chapter highlights principles and applications of different phage-based approaches.

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Figure 1.

Represents a variety of bacterial detection approaches, as well as their advantages (✓) and drawbacks (✗). These include culture and colony count, advanced molecular methods, and recent biosensors-based screening.
biosensors for the detection of pathogenic bacteria in clinical, food, and environmental samples.

2. Phage-based biosensors for infectious pathogen detection

Biosensors are nowadays omnipresent in point-of-care diagnosis as well as a wide range of other areas such as, environmental monitoring, food control, forensics, and drug discovery. They are devices that measure chemical or biological reactions by producing signals proportional to the concentration of an analyte in respective the reaction (Figure 2). The analyte is specifically recognized by a bioreceptor, such as cells, aptamers, nucleic acids, enzymes, as well as antibodies, and the conversion of the bio-recognition event into a measurable signal is carried out by a transducer [9]. On the other hand, bacteriophages, also simply known as phages, are viruses that infect bacteria, but are being harmless for all organisms including humans. During bacteriophage lytic cycle, the rupture of the host bacterial cell will result in releasing intracellular components, as well as in the liberation of virion progeny particles, which could be exploited as biomarkers for detection purposes [10].

Since bacteriophage particles are biological entities, capable of infecting specific bacterial hosts, they can be used as a bio-probe in different transduction platforms for pathogenic bacteria detection, which are briefed in the following sub-sections.

2.1 Phage optical biosensors

Detection using optical phage-based sensors is based on the variations generated in light properties, such as wavelength, polarization, refractive index [8]. Based on their working principles, optical methods are classified into two categories: label-free and labeled. Label-based biosensors detect changes in the presence of photons produced by optical labels at a particular wavelength. These could include DNA
intercalator dyes and fluorescent molecules. The label serves as an indirect marker of the presence of a specific analyte. Alternatively, label-free biosensors allow measurement of analyte physical properties without experimental ambiguity and enabling for more reliable analysis that involve minimal assay advancement [11]. These properties result in better sensitivity, rapid screening, and higher flexibility to a wide-ranging assay conditions. The most employed techniques for bacterial detection using optical biosensors are surface plasmon resonance (SPR), fluorescence spectrometry, and bio/chemiluminescence [8].

2.1.1 SPR-based sensors

Surface plasmon resonance (SPR) use plasmons, which are collective oscillations of electrons present at the surface of conducting materials. Thus, based on the principle of oscillation phenomenon that occurs between the interfaces of two materials, interactions between an analyte in solution and a recognition layer are monitored by a change in refractive index, which then lead to changes in the SPR angle of the reflected light [12]. Phages are designed as diagnostic bio-probes and immobilized on SPR transducer for specific detection of pathogens. For example, bacterial pathogens such as *E. coli* WG5 [13], methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli* O157:H7 [14], and *Salmonella typhimurium* [15] were successfully detected using this technique, where LOD (limit of detection) values were ranging from to $10^2$ to $10^3$ CFU/mL.

2.1.2 Fluorescent sensor

In these biosensors, phages are fluorescently stained and utilized as bio-indicators for the specific identification of bacterial cells, upon binding. The bacteriophage-bacteria interactions are evaluated by either flow cytometry or epi-fluorescent filter method [16]. In fact, it is reported that flow cytometry based, PP01 bacteriophage sensors have a sensitivity up to 1 CFU/mL for the detection of *E. coli* O157:H7 cells in apple juice samples [17]. While for epi-fluorescent microscopy-based approaches, the sensitivity was improved up to 20 CFU/mL, when fluorescent quantum dots (QDs) were utilized for phage labeling. These are semiconductor nanoparticles, which have been employed to visualize biological molecules in vitro and in vivo owing to their high photoluminance [18]. Bacterial toxins responsible for food poisoning such as staphylococcal enterotoxin B (SEB) can also be identified using fluorescent biosensors. Goldman et al. have developed a display phage to select peptides (12-mer) that are capable of binding to SEB. This approach has managed to detect 1.4 ng of SEB per sample well [19].

2.1.3 Bioluminescence sensors

For rapid, sensitive, and simple quantitative detection of bacteria in samples, bioluminescence analyses are applied by assessing emitted light from intracellular components e.g., ATP. These are based on the oxidation of organic compounds such as luciferin, by the enzyme luciferase, which then generates visible light in living organisms [8]. Biosensors based on this type of assay has managed to detect $10^3$ CFU/mL of *S. enteritidis* and *E. coli* G2–2 cells by specific lytic phages, SJ2 and AT20, respectively within a short period of 2 hours [20].

Also, it was demonstrated that recombinant luxAB-tagged reporter phage was able to detect around $10^5$ of *Yersinia pestis* cells in infected clinical specimens [21]. Other bacterial types that were successfully detected in food stuffs via
phage-mediated bioluminescence include Bacillus anthracis [22], E. coli O157:H7 [23], E. coli BL21 [24], and Listeria monocytogenes [25], and are briefed in Table 1.

### 2.2 Phage-based electrochemical biosensors

Electrochemical biosensors usually uncover electroactive species, following the variations of analyte electrical properties when undergoing redox reactions.

| Transducer     | Phage-based bio-probe | Target bacteria/analyte | Sample           | Detection limit | Refs. |
|----------------|-----------------------|-------------------------|------------------|-----------------|-------|
| SPR            | φX174                 | E. coli WG5             | PBS              | 10^7 PFU/mL     | [13]  |
|                | BP14 phage            | MRSA                    | PBS              | 10^7 CFU/mL     | [14]  |
|                | T4 phage              | E. coli O157:H7         | PBS              | 10^7 CFU/mL     | [14]  |
|                | P22 phage             | S. typhimurium          | LB broth         | 10^7 CFU/mL     | [15]  |
| Fluorescent    | PP01 phage            | E. coli O157:H7         | Apple juice      | 1 CFU/mL        | [17]  |
|                | λ (lambda) phage      | E. coli K12             | PBS              | 20 CFU/mL       | [18]  |
|                | M13 phage peptides    | SEB                      | PBS              | 1.4 ng/well     | [19]  |
| Bioluminescence| SJ2 phage             | S. enteritidis          | TSB              | 10^3 CFU/mL     | [20]  |
|                | AT20 phage            | E. coli G2–2            | TSB              | 10^3 CFU/mL     | [20]  |
|                | φA1122 phage          | Y. pestis               | Clinical matrix  | 10^3 CFU/mL     | [21]  |
|                | Wj::luxAb phage       | B. anthracis            | Food matrix      | 3.2 × 10^5 CFU/mL | [22] |
|                | phiV10lux phage       | E. coli O157:H7         | Ground beef      | 17 CFU/g, 10 CFU/cm² | [23] |
|                | T7 bacteriophage      | E. coli BL21            | Cheese           | 2.37 × 10^5 CFU/well | [24] |
|                | A511::Nluc phage      | L. monocytogenes        | Milk, lettuce   | 1 CFU/ 25 g     | [25]  |
| Amperometric   | B1–7064 phage         | B. cereus               | Nutrient broth   | 10 cells /mL    | [26]  |
|                | D29 phage             | M. smegmatis            | Nutrient broth   | 10 cells /mL    | [26]  |
|                | T7::ß-gal phage       | E. coli BL21            | Apple juice, drinking water, skim milk | 10^5 CFU /mL | [27] |
|                | Thi, T4 phages        | E. coli ATCC 11303      | LB broth, PBS    | 1 CFU /mL       | [28]  |
| Impedimetric   | Endolysin Ply500      | L. monocytogenes        | Milk             | 10^5 CFU/mL     | [29]  |
|                | Lytic phage           | S. arlettae             | Spiked water, apple juice | 2–2 × 10^6 CFU/mL | [30] |
|                | γ (gamma) phage       | B. anthracis            | Water            | 10^7 CFU/mL     | [31]  |
|                | M13 phage             | E. coli K12             | River water      | 14 CFU/mL       | [32]  |
They are noted for their robustness, low-cost, and simplicity. The key advantages of this type of biosensors are their relative sensitivity. Limitations could include low specificity from the interference of other redox-active species, as well as the requirement of mediators. Moreover, typical electrochemical approaches involve amperometric and/or potentiometric measurements. In fact, the first ever biosensor established by Clark and Lyons in 1962, was a simple form of an amperometric biosensor \[42\]. Generally, amperometric and impedimetric approaches are the most exploited detection techniques in the research involving phage-based electrochemical biosensors.

### Phage-amperometric biosensors

Amperometric detection involves the constant measurement of a current resulted from the reduction or oxidation of electrolytes in a biochemical reaction, taking place between the working (having bio-probe) and reference electrodes. Thus, for current production in the analyte sample, a bias potential is passed on these electrodes, and the generated current is directly proportional to the ionic changes in analyte’s concentration \[43\]. Built on this principle, it was reported that bacteria like \textit{Bacillus cereus} and \textit{Mycobacterium smegmatis} were successfully detected due to enzymatic release by bacteriophage lysis, in which this enzymatic activity is measurable in a particular substrate \[26\]. For the detection of \textit{E. coli} using the T7 phages, Wang et al. proposed a promising biosensor that had provided a limit of

| Transducer       | Phage-based bio-probe | Target bacteria/analyte | Sample          | Detection limit | Refs. |
|------------------|-----------------------|--------------------------|-----------------|-----------------|------|
| QCM              | Filamentous phage     | \textit{S. typhimurium}  | PBS             | \(10^7\) CFU/mL | \[33\] |
|                  | Lytic phase 12600, PBP 2a Ab | \textit{S. aureus}, MRSA | Water           | —               | \[34\] |
|                  | E79 phage             | \textit{P. aeruginosa}   | LB broth        | \(1.5 \times 10^5\) bacteria \(\text{cm}^2\) \(\text{Hz}^{-1}\) | \[35\] |
|                  | Phage-displayed mimotope, Ag8SB | \textit{M. leprae}  | Clinical samples | Low-titer Ab in patients | \[36\] |
| Magnetoelastic    | JR87 phage            | \textit{B. anthracis}    | PBS             | —               | \[37\] |
|                  | Lytic phage           | \textit{MRSA}           | —               | \(10^7\) CFU/mL | \[38\] |
|                  | E2 phage              | \textit{S. typhimurium}  | Tomatoes        | \(5 \times 10^5\) CFU/mL | \[39\] |
|                  | E2 phage              | \textit{S. typhimurium}  | Eggs            | \(1.6 \times 10^5\) CFU/cm\(^2\) | \[40\] |
|                  | E2 phage              | \textit{S. typhimurium}  | Spinach leaves  | 100 CFU/25 g    | \[41\] |

\(\text{Ab, antibody; SEB, staphylococcal enterotoxin B; SPR, surface plasmon resonance; MRSA, methicillin-resistant Staphylococcus aureus; PBS, phosphate-buffered saline; QCM, quartz crystal microbalance; QD, quantum dot; CFU, colony-forming unit; FFU, plaque-forming unit; E. coli, Escherichia coli; S. arlettae, Staphylococcus arlettae; B. cereus, Bacillus cereus; B. anthracis, Bacillus anthracis; M. smegmatis, Mycobacterium smegmatis; M. leprae, Mycobacterium leprae; P. aeruginosa, Pseudomonas aeruginosa; S. typhimurium, Salmonella typhimurium; S. aureus, Staphylococcus aureus; LB, Luria-Bertani; Thi, thionine; TSB, Trypticase Soy Broth; ng, nanogram; PBP 2a, penicillin-binding protein; Y. pestis, Yersinia pestis.}
detection as $10^5$ CFU/mL in 3 hours and $10^2$ CFU/mL after 7 hours in aqueous samples, such as apple juice, drinking water, and skim milk. The team have engineered a T7 phage in conjunction with $\text{lacZ}$ operon encoding for $\beta$-galactosidase ($\beta$-gal), which have led to the overexpression of $\beta$-gal and the release of a huge amount of the enzyme biomarkers during the infection of $E. \ coli$ BL21 cells and their lysis (Figure 3). Then, the phage-produced $\beta$-gal will be detected with 4-aminophenyl-$\beta$-galactopyranoside (PAPG) as a substrate. The product $p$-aminophenol ($p$-AP), which was monitored by amperometry as electrochemical signal, was directly proportional to the bacteria concentration in the tested sample [27].

In other cases, phages are used as recognition probes instead of as a tool for specific detection of released cell content. For example, Li et al. developed a similar approach for the detection of $E. \ coli$ cells in urine samples, where phage-coated organic–inorganic hybrid nanoflowers were utilized as the detection probe, and the AMP magainin, I as the capture probe. The detection probes were constructed by mixing gold nanoparticles (GNPs), nanoflowers, as well as thionine (Thi) and T4 bacteriophages. The latter served as the signal amplification step due to capacity of catalyzing three cascade redox reactions in working solution. This method has provided a very low detection limit as 1 CFU/mL [28].

2.2.2 Phage impedimetric sensors

In addition to amperometry, impedimetric techniques such as electrochemical impedance spectroscopy (EIS) are also employed in bacteriophage-based sensors for detection of bacterial pathogens. In such approach, the infection of target bacteria cells by phages immobilized on the surface of working electrode causes a change in impedance via an ‘insulating’ behavior, which can be measured [8]. The integration of endolysin-coding bacteriophages and EIS-based platform was reported for the detection of $L. \ monocytogenes$ bacterial cells in milk samples by ply500 phage immobilization on the surface of gold screen printed electrode (SPE) electrode with LOD of $\sim 10^5 \text{ CFU/mL}$ [29]. Moreover, SPE electrodes can be made of other materials with similar properties. These include graphene, which proved effective in the recognition of $Staphylococcus arlettae$ using specific lytic phages.
These were immobilized on the sensor’s surface for the quantitative analysis of the bacterial cells for a broad detection range of $2 - 2 \times 10^6$ CFU/mL. Accordingly, the lowest limit of detection recorded was defined as 2 CFU/mL [30]. Moreover, carbon SPE electrodes were successfully employed in the detection of *Bacillus anthracis* in aqueous electrolyte media using *Gamma* phages as probes [31]. This approach can be improved by depositing gold nanoparticles on the surface of glassy carbon electrode, in which their high surface area would allow efficient chemical binding of phages. Sedki et al. have immobilized a non-lytic M13 phage on this type of electrodes by means of 3-mercaptopropionic acid as a linker. The biosensor showed an outstanding stability over a wide range of temperatures (25 and 45°C) and pH levels (3.0–10.0), providing a very promising LOD of 14 CFU/mL. In addition, the detection was selective, and the specificity was confirmed by using *Pseudomonas chlororaphis* as a negative control [32].

2.3 Phage-based mass sensitive sensors

Also known as ‘piezoelectric’ biosensors, these work on the principle of affinity interaction. This means that any change in oscillations due to mass bound on the surface of a piezoelectric crystal, would produce an electrical signal when a mechanical force is applied [44]. Well-known examples of mass-based biosensor include the quartz crystal microbalance (QCM) platform and magnetoelastic sensors (MES).

2.3.1 Phage-QCM-based sensors

Quartz crystal microbalance (QCM) biosensors are highly sensitive mass-based sensors, owing to their capacity of detecting very low variations in mass e.g., nanograms. Here, both sides of a thin piezoelectric film are coated with two conductive electrodes. When an electrical field is applied through the quartz crystal, mechanical resonance is stimulated, and mass of target analyte is quantified [45]. Thus, phages can be conjugated as bio-probes on surface of QCM sensors for selective screening of bacteria. For instance, filamentous bacteriophages immobilized on the surface of piezoelectric transducer offered a very sensitive and rapid identification of *S. typhimurium*, with a LOD of $10^2$ CFU/mL, and under 3 minutes reaction time [33]. These results and the quality of phage deposition were further confirmed by fluorescent and scanning electron microscopy (SEM). Other reports of QCM-based phage sensor applications in bacterial detection are briefed in Table 1.

2.3.2 Phage magnetoelastic sensors

Magnetoelastic sensors (MES) are a class of wireless biosensors that are fabricated from materials with specific characteristics, such as magnetism and elasticity. Thus, the fundamental operating principle entails a change in resonance frequency because of mass loading of the sensor, which is associated with binding of an analyte to a bio-receptor immobilized on the sensor’s surface. Magnetoelastic devices are being developed for the on-site and real-time detection of pathogenic bacteria by integrating phages as bio-probes [46]. Likewise, *B. anthracis* spores [37] were specifically detected using JRB7 filamentous phage, yielding a sensitivity value of 202 Hz/decade. Moreover, the authors have demonstrated that 420 mM salt at a phage concentration of $1 \times 10^{11}$ viruses/mL have resulted in an optimal distribution of immobilized phages on the sensor’s surface, consequently promoting better binding of spores to the biosensor’s surface (Figure 4). Lytic phages have been also proven as promising bio-probes for the detection of MRSA with LOD of $10^3$ CFU/mL.
Their enhancement required optimal conditions of 30 minutes of immobilization time and a bacteriophage concentration of $10^{11}$ PFU/mL [38].

In addition, several reports have emphasized on the outstanding specificity of E2 phage-coated MES devices in the detection of *S. typhimurium*, in various food samples such as tomatoes [39], eggs [40], and spinach leaves [41], as well as in environmental samples such as soil [47]. In these studies, the immobilized, filamentous E2 phages were selected from a landscape f8/8 phage library and genetically engineered for the biorecognition of the bacterium. In a nutshell, the binding of E2 phage and *S. Typhimurium* raises the total mass on the sensor, so its vibration is reduced, which leads to a resonant frequency shift directly proportional to number of bacteria bound to MES. Furthermore, E2 phage-based magnetoelastic biosensors expressed incredible stability when exposed to harsh environmental conditions [48].

3. Applications of phage-based biosensors

Despite the above-mentioned applications of bacteriophage-based sensors in detection of clinical pathogenic bacteria, food safety, and environmental monitoring (Table 1), the following section highlights some other representative applications of phage-based biosensors in detection of bacterial pathogens in agriculture, as well as in wastewater management.

3.1 Agriculture

The use of phages in agriculture and aquaculture is an upcoming area of development. Bacterial infection in crops is a serious problem that reduces the yields. To diagnose crop pathogens associated with wilt and blight, a limited number of research and development studies have applied phage-based sensing platforms [49]. Phage-based biosensors have been fabricated for *Pseudomonas*, *Erwinia*, and *Ralstonia* spp. In fact, *Pseudomonas cannabina* pv. *alisalensis* is a causative agent of blight *Brassicaceae*, which are nutritious cruciferous vegetables that are very...
important for oil seed and energy crops [50]. To identify this pathogen, a group of authors established a bioluminescent sensor that can differentiate it from *Pseudomonas syringae*, a different plant pathogen, with a LOD of $1.3 \times 10^3$ CFU/mL. The assay was founded on a luciferase recombinant phage PBSPCA1 [51]. Similarly, *Erwinia amylovora* infects *Rosaceae* plants, comprising fruits such as pears and apples, causing fire blight. Compared to ELISA-based methods, an engineering Y2 phage that also uses luciferase has offered a successful detection of *E. amylovora*, with enhanced sensitivity and specificity, yielding a LOD of $3.8 \times 10^3$ CFU/mL [52]. In case of *Ralstonia solanacearum* identification, a devastating phytopathogen with a wide host range of over 50 species tropical agricultural crops, another biosensing approach was used. The authors reported a less destructive and more sensitive method, which have involved phage amplification in combination with real-time PCR (RT-PCR). The limit of detection was found to be $10^2$ CFU/g in leaf and soil samples, after an hour of reporting time [53].

Nevertheless, this field of research is ripe for prolonged investigation to lower diagnostic costs and reporting times within the agricultural industry.

### 3.2 Wastewater management

The diagnostic methods that have been described using phages to target pathogens in water are limited. However, the ability to detect bacterial pathogens directly from water offers an exceptional opportunity to evade pre-enrichment steps [54]. According to safety regulations by World Health Organization (WHO), no detectable coliforms should be present in 100 mL of drinking or crop rinsing water. For example, *E. coli* concentration in recreational water must be less than 100 cells per 100 mL. Therefore, minimal infectious dose (MID) of pathogenic bacteria is a crucial factor that must be considered when monitoring environmental sources for outbreaks [55]. In theory, it can be considered as the minimum required sensitivity of any adopted monitoring method [56]. Among the most problematic water-borne pathogenic bacteria, *E. coli* and *Vibrio cholerae* may cause severe symptoms such as hemolytic uremia and hemorrhagic colitis, as well as diarrhea and gastroenteritis, respectively [57].

For *E. coli* BL21 detection in drinking water, researchers have recently developed a syringe-based biosensor, where the genetically modified T7 phage combined membrane filtration with selective enrichment. The assay has managed to detect 20 CFUs in 100 mL of water, within five hours. The increased selectivity and sensitivity were mainly due to innovative combination of membrane filtration with phage infection [58]. Similarly, other water-borne bacterial pathogens such as *P. aeruginosa*, *V. cholerae*, and *Xanthomonas campestris* were detected in tap and seawater samples, with assay time of an hour, LOD of $\sim100$ cells, and showing no cross-reactivity. The colorimetric sensor involved an engineered M13 bacteriophages, which expressed receptor-binding proteins (RBPs) with thiolated capsid that allows gold nanoparticles (AuNPs) attachment, resulting in color change [59]. By this straightforward approach, sensitive identification of bacteria in situations where time and/or equipment resources are limited, could be possible and advantageous.

### 4. Conclusions and remarks

Certainly, and without a doubt, food safety and environmental monitoring must be taken into consideration when fighting infectious pathogens and limiting their dissemination. In this chapter, the main applications of recently developed platforms of phage-based biosensors, in the screening of human food- and water-borne bacteria, were evidently demonstrated. Moreover, different bacteriophages and their
components applied in the development of sensors were reviewed, demonstrating their usefulness in diagnosing various food contaminants as well as their toxins, compared to currently used conventional methods. However, in addition to the cost of bacteriophages and the relevant reagents, their purification is still laborious and thus expensive from the commercial perspective. For this reason, more efforts are required in enhancing phage-based materials, as well as processing time and limit of detection. The collaboration between engineers and researchers from multidisciplinary fields such as biology, chemistry, engineering, and electronics will therefore help design more advanced phage-based sensors for food safety and environmental monitoring. In summary, applications of bacteriophage sensors in the fields of clinical diagnosis, food safety, and environmental monitoring are crucial.

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Acronyms and abbreviations

| Abbreviation | Description |
|--------------|-------------|
| Ab           | antibody    |
| ATP          | adenosine triphosphate |
| AuNPs        | gold nanoparticles |
| β-gal        | β-galactosidase |
| CDC          | Centers for Disease Control and Prevention |
| CFU          | colony-forming units |
| DNA          | deoxyribonucleic acid |
| E. coli      | Escherichia coli |
| EIS          | electrochemical impedance spectroscopy |
| ELISA        | enzyme-linked immunosorbent assay |
| GNP         | gold nanoparticles |
| LB           | Luria-Bertani broth |
| LOD          | limit of detection |
| MES          | magnetoelastic sensors |
| MID          | minimal infectious dose |
| MRSA         | methicillin-resistant staphylococcus aureus |
| NA           | nucleic acid |
| PBS          | phosphate-buffered saline |
| PBP2a        | penicillin-binding protein |
| PCR          | polymerase chain reaction |
| PDPs         | phage-display peptides |
| PFU          | plaque-forming unit |
| QCM          | quartz crystal microbalance |
| QDs          | quantum dots |
| RBP          | receptor-binding proteins |
| RT-PCR       | real-time PCR |
| SEB          | staphylococcal enterotoxin B |
| SEM          | scanning electron microscopy |
| SPE          | screen printed electrode |
| SPR          | surface plasmon resonance |
| TSB          | trypsinase soy broth |
| WHO          | World Health Organization |
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