Bacteriophage Tail Proteins for Bacterial Pathogen Recognition

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In recent years, a number of bacterial detection methods have been developed to replace time-consuming culture methods. One interesting approach is to mobilize the ability of phage tail proteins to recognize and bind to bacterial hosts. Bacteriophages combine several properties that are desirable for the purpose of detecting bacterial pathogens. Compared to the traditional culture-based methodologies, capture-dependent methodologies are accurate, reliable, simple, relatively inexpensive, fast, and require a fairly low skill level. These properties are all desirable for diagnostics, suggesting that phage tail protein-based capture methods could potentially improve the treatment and control of pathogenic bacteria, thereby decreasing their negative impact worldwide.

Keywords: tail fiber protein ; pathogens detection ; bacteriophages

1. *Acinetobacter baumannii* Detection

Acinetobacter baumannii, considered to be one of the opportunistic pathogens, is notorious for its propensity to cause nosocomial infections, including sepsis and ICU (Intensive Care Unit)-acquired pneumonia [1]. It is one of the ESKAPE pathogens, which are a group of highly virulent bacteria with high antimicrobial resistance that are able to “escape” the harmful effects of antibiotics and cause serious infections [2][3]. The success of this pathogen has further been attributed to its outstanding ability to withstand harsh environmental conditions. This is achieved in part through surface glycans such as CPS (capsular polysaccharide), which is composed of multiple repeating oligosaccharide units [4]. The CPS structure called the K type has been elucidated for multiple *A. baumannii* isolates, but no comprehensive serotype classification has been developed to date [5]. The extraordinary diversity of the *A. baumannii* CPS is reflected by the existence of at least 128 different gene clusters (KL clusters) responsible for CPS synthesis and export [6]. Importantly, CPS is targeted by phage RBP during adsorption [7]. Increasing evidence suggests that the K type is an important specificity determinant, with many *A. baumannii* phages infecting strains of a single or very limited number of K types [8][9][10][11][12].

Many groups have studied *A. baumannii* phage tail fiber/tailspike proteins with a particular focus on their depolymerase activity and potential as a prospective antivirus treatment [13][14][15][16][17][18]. Below, researchers attempts to harness the specificity of RBPs for detecting *A. baumannii.* The extensive diversity of CPSs in this pathogen limits the feasibility of a method aimed at its rapid and broad-range detection. A more feasible application for phage RBPs in this case might be using them to develop a typing scheme. Xu et al. (2020) recently described the isolation of a new phage capable of infecting five strains of *A. baumannii*. The authors identified two putative tail fiber proteins (TFPs) in genomes of these phages and demonstrated that they bind susceptible cells and lack lytic activity, and thus could be useful as detection tools. To examine the target specificity of the TFPs, the authors devised a sandwich fluorescent assay. Microplate wells were coated with non-labeled TFPs to capture bacterial cells, and bacteria were subsequently detected using analogous, FITC (fluorescein isothiocyanate)-labeled proteins and fluorescence microscopy. The probes exhibited good specificity, as they did not recognize selected strains of *E. coli*, *Acinetobacter haemolyticus* or *Bacillus subtilis*, but they did recognize a susceptible *A. baumannii* strain in a mixed culture. For a detection assay, bacteria were incubated with TFPs conjugated to magnetic beads and the results were visualized using a bioluminescence assay with a commercial luciferin/luciferase kit that measured intracellular ATP release. The authors claimed that an optimized protocol enabled them to detect as few as 6.2×10^2 CFU/mL (colony-forming unit per mililiter) *A. baumannii*. They investigated the clinical applicability of this technique by testing artificially contaminated human samples (urine, sputum, feces), and found that 50–93% of the target bacteria could be recovered from the sample, depending on the sample type and bacterial load [19].

Bai et al. (2019) reported an approach in which alumina-coated magnetic nanoparticles were used to immobilize two distinct TFPs (TF2 and TF6) that selectively recognized two clinical strains of *A. baumannii*. Binding efficiency was evaluated in terms of the change in optical density (OD600) between readouts taken before and after incubation with a

nanoparticle probe. The OD change was significantly higher for target bacteria than for non-target bacteria. TEM (transmission electron microscopy) observations showed significant aggregation of nanoparticles, but their binding to bacteria was also apparent.

For the detection of *A. baumannii*, the authors combined nanoparticle trapping with MALDI-TOF MS (matrix-assisted laser desorption-ionization time-of-flight mass spectrometry) analysis to uncover a protein fingerprint for further identification. With this approach, the two *A. baumannii* strains were distinguished from one another as well as from other pathogens (*E. coli* O157:H7 and *Staphylococcus aureus*) in a mixed sample. Additionally, the TFP-coated nanoparticles allowed the sample to be enriched to a concentration of bacteria detectable by MALDI-TOF MS. The level of detection for the method was 2.34×10^5 or 4.48×10^4 CFU/mL, depending on the strain. The method was able to detect low concentrations of bacteria (5×10^5–2×10^6 CFU/ml) in diluted fetal bovine serum, supporting its clinical potential. The authors did not examine lower bacterial concentrations, presumably because high ion peaks resulting from the decaying probes would easily obscure the peaks from bacteria present in a sample at a lower concentration. However, MALDI alone, without the selective enrichment, did not detect bacteria present at such a concentration.

To further demonstrate the selectivity of TF2-coated nanoparticles, the authors examined 10 clinical strains: four strains that had been previously described to bind TF2 and six non-binding strains. Bound cells were recovered from the nanoparticles and the CFU of each strain per mg of protein was compared to the value for the original target strain. At a cut-off value established by the authors (≥70% recovery indicating a positive result, <70% indicating a negative result), TF2-coated nanoparticles could differentiate between known TF2-binding and non-binding strains. [20].

The *A. baumannii* ΦAB6 phage tailspike protein, TF6 (ΦAB6TSP), has attracted attention due to its ability to recognize and hydrolyze bacterial surface glycans containing pseudaminic acid (Pse) [21]. Pse has been identified in numerous Gram-negative pathogens, including *Campylobacter jejuni* and *Helicobacter pylori*, and it has commonly been implicated in virulence [22]. In *A. baumannii* strains, the sequence encoding Pse was identified in two capsule biosynthesis gene clusters [23]. The use of this particular TSP could, in principle, allow the detection of various Pse-coated bacteria. This was recently investigated by Lee et al. (2020). Because the enzymatic activity of Pse could hinder downstream detection, the authors prepared an inactive mutant protein that retained its target specificity. The corresponding inactivated fluorophore-conjugated TSP exhibited a strong relationship between signal intensity and probe/bacterial concentration. The TSP-based probe was deemed to be more sensitive in detecting a susceptible *A. baumannii* strain than an antibody raised against EPS (exopolysaccharide) hydrolysis products containing Pse. The authors also attempted the detection of other Pse-coated bacteria (two strains of *H. pylori* and one of *Enterobacter cloacae*). These strains showed a similar binding capacity, as reflected by fluorescence microscopy observations and the identification of a linear relationship between the fluorescence intensity and optical density of bacterial suspensions. The authors further developed an assay for detecting Pse-coated bacteria by immobilizing the TSP onto the wells of a microplate and measuring the fluorescence of FITC-labeled, bound bacteria. They observed a linear relationship between the amount of bacteria and the fluorescence intensity for Pse-containing strains, but not for Pse-lacking strains. However, the authors noted that the labeling and immobilization steps can introduce significant variation in the results, limiting the usefulness of this technique [24].

2. *Campylobacter spp.* Detection

Campylobacter spp. is a Gram-negative foodborne bacterial pathogen. The first report of *Campylobacter* spp. dates back to 1886, when Theodore Escherich observed non-culturable spiral-shaped bacteria. The hosts of this pathogen include both wild and domestic animals; it is frequent among birds, especially poultry, probably because of their higher body temperature [25]. Chicken products have been implicated in a large number of *Campylobacter* spp. infections in human populations, due to the high consumption of chicken meat [26][27][28][29]. *Campylobacter* spp. is also commonly found in other livestock, such as cattle, pigs, cows, lambs, ducks, and turkeys [30]. Consumption of untreated water is also considered a risk factor [26][31]. *Campylobacter* spp. causes mild to serious infections of children and the elderly, called campylobacteriosis. The most common symptom is diarrhea, but the infection may lead to permanent neurological damage, such as that of Guillain–Barré syndrome [26]. For *Campylobacter* and certain other species, viable but non-culturable cells (VBNC) can exist (but not replicate) under unfavorable growth conditions. Cells in this state can still infect susceptible hosts [26][32].

Poshtiban et al. (2013) proposed a platform that used the bacteriophage tailspike protein, GST-Gp48, to detect pathogenic *C. jejuni* via an interesting biosensor-based method [33][34]. They employed a micromechanical resonator that enabled the high-throughput and label-free diagnostic analysis of multiple samples. During the experiment, the device monitors the resonance frequency shift, which depends on the mass of the adsorbed target analyte. The microresonator was functionalized with the GST-Gp48 tailspike protein, and the specific capture and detection of *C. jejuni* cells was

demonstrated. This microresonator array was highly mass sensitive and had a large surface-to-volume ratio. The detection and quantification of *C. jejuni* cells were confirmed by an SEM (Scanning Electron Microscope). The simulations of resonance behaviors were tested using Finite Element Analysis (FEA). The results of these simulations showed that the frequency shift was determined by the mass of bacteria captured on the microresonator surface. The authors used SEM visualization to calculate the number of bacteria attached to the sensor and found that 225 ± 13 bacteria bound to a single element on average. The specificity of capture was established by comparing binding between *C. jejuni* and *E. coli* cells (negative control). The findings were promising, and the method seemed to be relatively inexpensive and rapid compared to conventional methods. This microresonator-based biosensor enabled the highly specific detection of bacteria in a sample with a low bacterial load. Moreover, microresonator arrays do not require sample pre-enrichment and the experiments are label-free [33].

Two methods for detecting *C. jejuni* and *Campylobacter coli* were reported by Javed et al. (2013). The authors described a phage RBP-based agglutination assay and a method of GFP-coupled RBP probe detection combined with fluorescence microscopy. First, the team identified an RBP (Gp047) from *C. jejuni* phage NCTC12673 and established that its C-terminal domain was responsible for its specific host recognition. They found that *C. jejuni* NCTC11168 cells mixed with CC-Gp047 formed aggregates within 1 min on a glass slide. The authors confirmed that the observed aggregates were caused by probe binding and not by autoagglutination of bacterial cells. The agglutination test was performed on a wide range of bacterial species (*C. jejuni*, *C. coli*, *Campylobacter lari*, *Campylobacter fetus*, *Campylobacter fetus venerealis*, *Campylobacter concisus*, *Campylobacter upsaliensis*, *H. pylori*, *E. coli*, and *Salmonella enterica*). The results indicated that agglutination also occurred efficiently when the cells were in the VBNC state. To check the robustness of the method under different conditions, different growth media (MH, BHI, LB, and NCZYM) and buffers (phosphate buffer pH 7.4, HEPES buffer pH 7.4, standard saline, Tris-HCl buffer, pH 7.5, and 5% BSA in PBS) were examined. The agglutination results were the same for all tested conditions. The RBP-based agglutination assay showed 100% specificity for both *Campylobacter* species, and 95% and 90% sensitivity for *C. jejuni* and *C. coli*, respectively. Importantly, agglutination tests are rapid and do not require sophisticated equipment (only a simple glass slide) [35].

For the second method, the authors used GFP-fused CC-Gp047 and fluorescence microscopy for detection. Bacterial suspensions (*C. jejuni* NCTC11168, *C. coli* RM2228, and *E. coli* DH5α) were incubated with the labeled protein and microscopic observations were carried out. *C. jejuni* and *C. coli* showed green fluorescence, indicating the binding of EGFP_CC-Gp047, while *E. coli* DH5a did not. As a follow-up experiment, the authors tested a mixed culture of bacteria containing *C. jejuni* NCTC11168 and *E. coli* DH5a at a cell number ratio of approximately 1:25. Only *C. jejuni* cells (as discerned by characteristic morphology) showed green fluorescence under microscopic observation. The RBP-based probe, EGFP_CC-Gp047, combined with fluorescence microscopy was thus able to detect low numbers of *C. jejuni* and *C. coli* cells in a mixed culture. Both of the introduced methods are simple and specific and can be used for the simultaneous detection of pathogenic *Campylobacter* species [35].

3. *Listeria monocytogenes* Detection

Listeria monocytogenes is a major public health concern in the food industry due to its ability to survive the harsh conditions commonly applied in food processing and preservation, such as low temperature, high salt concentration, dehydration, and extreme pH [36]. Infection with *L. monocytogenes*, known as listeriosis, occurs as a result of ingesting contaminated foods; reported outbreaks of listeriosis have been mainly connected to dairy products, meat, fish, fruits, and vegetables [37]. Listeriosis generally presents as gastroenteritis, but in the case of vulnerable individuals (including children, the elderly, pregnant women, and immunocompromised individuals), it poses a threat of sepsis, meningitis, and premature termination of pregnancy [38]. Given the severity of symptoms in the susceptible population and the finding of a lower infectious dose than previously suspected, the U.S. Food and Drug Administration (FDA) adopted a “zero-tolerance policy” for this pathogen in ready-to-eat foods [39]. In contrast, the current EU (European Union) regulations accept a tolerable limit of 100 CFU/g for foods that do not support the growth of *L. monocytogenes* as they enter the market, as well as those that do support the growth of this pathogen over the product’s shelf life [40].

Currently, the detection of *L. monocytogenes* and other *Listeria* spp. in food samples is commonly based on culture methods [36]. As a general rule, one or two enrichment steps (i.e., incubation in a selective liquid medium) are required to enrich the target bacteria to a detectable concentration and inhibit the growth of competing microflora. Cultures are then plated onto selective differential or chromogenic media and the resulting colonies are examined for characteristic morphology. As a slow-growing microorganism, *Listeria* spp. requires extended incubation times, making this a relatively slow process. Thus, new, rapid, and robust detection methods are needed. Numerous nucleic acid-based techniques have been applied to the detection of *L. monocytogenes*, including PCR, multiplex PCR, real time/quantitative PCR (qPCR), loop-mediated isothermal amplification (LAMP), and whole-genome sequencing analyses. For a more detailed

description of representative culture methods and novel molecular approaches, the interested reader is referred to the review by Law et al. (2015). These molecular techniques are extremely sensitive and specific; however, they are generally unable to discriminate between viable and inactivated microorganisms, resulting in false positives [41]. Meanwhile, the harsh conditions of food processing are expected to stress bacterial cells, causing them to become non-cultivable on selective media and leading to their underestimation in culture-based methods [42].

L. monocytogenes is currently classified into 12 serotypes; of them, serotypes 1/2a, 1/2b, and 4b are the most commonly associated with clinical disease [43]. The serotypes are determined primarily by the structure of the cell wall-associated carbohydrate polymer, teichoic acid, which is specifically recognized during phage adsorption by RBP [44]. Phages of Gram-positive hosts, including *L. monocytogenes*, possess endolysins (peptidoglycan-cleaving enzymes) with CBDs that exhibit remarkable specificity towards peptidoglycan-associated molecules, including teichoic acid [45][46]. The selectiveness of both types of phage-derived proteins supports their possible application in diagnostic and/or serotyping tools.

The phage-based methods reported to date for detecting *L. monocytogenes* have focused primarily on endolysins [47][48][49][50] and broad-range *Listeria* reporter phages [51][52][53]. A commercially available rapid and semi-automated phage protein-based test for detecting *Listeria* spp. was developed and validated (VIDAS UP Listeria, bioMérieux) [53]. Based on the principle of enzyme-linked fluorescent assay, this method uses alkaline phosphatase-conjugated protein probes to capture target bacteria onto a solid surface and direct substrate cleavage to enable fluorescence detection [54]. However, the exact identity of the utilized phage proteins has not been disclosed.

The applicability of phage TFP and endolysins for the diagnosis and typing of *L. monocytogenes* was recently demonstrated by Sumrall et al. (2020). The authors gathered a set of six proteins (three RBP and three endolysin-derived CBD) from a collection of *Listeria* phages with the aim of developing a quick, reliable, and objective serotyping method. In this scheme, GFP-tagged recombinant proteins were incubated with bacteria on a microplate and the fluorescence from bound probes was measured to provide a serovar-specific fingerprint in ~1 h. To verify the accuracy of this novel glycotyping scheme, the authors tested 60 strains of known serotypes; of them, 58 were correctly assigned by the method. Unlike traditional serotyping with a slide agglutination test, which relies on the polyvalent binding of antibodies in serum, this technique relies on precisely described interactions with specific sugar moieties. Importantly, it eliminated the potential discrepancies that could be caused by the subjective interpretation of results or the use of serum preparations from different batches. It also circumvented some of the limitations of PCR-based typing, as it differentiated between live and inactivated bacteria as well as between closely related serotypes arising from a single point mutation.

To further illustrate the usefulness of the phage protein-based detection and differentiation of *L. monocytogenes*, the authors developed a method for differentially separating the most common pathogenic serotypes: 1/2 and 4b. Paramagnetic beads were functionalized with one of two selected GFP-tagged RBPs to allow for the selective enrichment and detection of these serotypes under fluorescence microscopy. The probe-bound beads were found to be efficient at separating the target serotypes in a mixed culture, although the binding affinity of the 4b-specific beads was significantly lower than that of the 1/2-specific ones. This finding led the authors to develop avidin-tagged directionally coupled probes that exhibited significantly better affinity but lacked the GFP tag essential for fluorescence-based detection. When tested against a comprehensive library of strains of different serotypes, both probes exhibited good specificity. Surprisingly, the 4b-targeting beads also bound nonpathogenic *Listeria innocua* serotype 6a and *L. monocytogenes* 4e, even though the latter harbored few target glycan molecules. These results suggest that phage tail proteins could be a useful tool for developing a rapid and reliable diagnostic assay for *L. monocytogenes* [44].

A different strategy for detecting *Listeria* spp., *E. coli* O157:H7, and *Salmonella* spp. was implemented by Junillon et al. (2012). The authors developed a simple device comprising a polystyrene surface coated with phage proteins and a colorimetric reaction to visualize bound bacteria. The detection was carried out directly in homogenized food samples over the course of a standard enrichment-period incubation (22–40 h depending on the strain) in a liquid medium. The visualization was based on the reduction of colorless triphenyltetrazolium chloride. The resulting red formazan crystals accumulated inside the cells, giving the sensor surface a colored appearance upon binding. To test the applicability of this method, the authors incubated homogenates with approximately 5 CFU of a given strain in the presence of the detection device. The test gave a strong positive result for *L. monocytogenes* serotype 4b and *Listeria seeligeri* in roast pork. Of note, this test used a broad-range phage protein and thus did not discern between different *Listeria* species [55].

4. *Yersinia pestis* Detection

Yersinia pestis is a highly pathogenic Gram-negative bacterium that is the causative agent for plague. Historically, this serious and potentially deadly zoonotic disease was responsible for pandemics occurring in the mid-6th, mid-14th, and early 20th centuries [56][57]. *Y. pestis*, which is a member of the *Enterobacteriaceae* family, is a nonmotile, non-spore-forming coccobacillus. Its growth temperature ranges from 4 to 40 °C, with an optimum range of 28–30 °C. The high pathogenicity of this bacterium reflects its ability to adapt to temperature changes: it can survive and replicate in both cold-blooded insects (fleas with body temperature of 20–28 °C) and warm-blooded mammals. Moreover, the bacterium can form a gel-like protective capsule with antiphagocytic properties. Humans can become infected after being bitten by a rodent-hosted flea or by direct contact with animals suffering from plague. Three clinical forms of plague can be distinguished: bubonic, pneumonic, and septicemic. Pneumonic plague is the most serious form of the disease, and the only one that can be transmitted from person to person. Due to its highly contagious nature, *Y. pestis* is currently detected with the following methods: ELISA (enzyme-linked immunosorbent assay)-based antigen detection, culture-based identification, F1 capsule antigen detection, PCR amplification, and virulence gene detection [57]. RBP proteins from *Y. pestis* phages φA1122 and L-413C were coupled to a fluorescent reporter protein to create a specific fluorescent probe for detecting bacterial cells under fluorescence microscopy. The initial observations confirmed that both tested proteins bound to *Y. pestis* cells after only 20 min of incubation. The authors then examined how culture temperature influenced the efficiency of binding. The results indicated that after 2 h incubation with bacteria in early logarithmic phase, RBP binding was observed at all tested temperatures (6, 20, 28, and 37 °C). However, the fluorescent signals for both RBPs were significantly weaker at 6 °C than at higher temperatures (20–37 °C for phage L-413C RBP and 28–37 °C for phage φA1122 RBP) [57]. The specificity of this test was confirmed using *Y. pestis* and other *Yersinia* species as a control, and the assay was performed under different growth and capsule-inducing conditions [57].

5. Pseudomonas aeruginosa Detection

Pseudomonas aeruginosa is a significant problem in healthcare systems. Infections caused by this bacterium are problematic in ICUs and are associated with high morbidity and mortality. This pathogen is especially dangerous for people with pneumonia, chronic obstructive pulmonary disease, or cystic fibrosis. The World Health Organization put this microorganism on the priority list of bacterial pathogens for which the development of new drugs is urgently needed [58]. This bacterium is responsible for 10–15% of nosocomial infections worldwide [59]. Additionally, infections caused by *Pseudomonas* are difficult to treat due to antibiotic resistance and the ability of this pathogen to acquire resistance to different antimicrobial agents [60]. *P. aeruginosa*-related bloodstream infection, which is considered to be one of its most serious complications, has a mortality of 18–61% [61]. Efforts to develop rapid diagnostic tests for pathogen recognition is an important element of the fight against *P. aeruginosa*-induced infections. One proposed method used a recombinant phage tail fiber protein (P069) from phage PA1. P069 was composed of wild-type TFP, a six-histidine (6-His) tag at the C-terminus, and three lysines at the N-terminus. To confirm the detection of bacteria by interaction of the obtained fluorescent protein, the authors performed TRITC (tetraethyl rhodamine isothiocyanate) labeling. The interaction of *P. aeruginosa* PA1 with the fluorescently labeled protein was observed under fluorescence microscopy. P069 was further functionalized with AffiAmino magnetic particles and incubated with *P. aeruginosa* to show the potential of magnetic separation as a tool for specific bacterial detection. The usefulness of P069 for efficiently detecting *P. aeruginosa* was confirmed in human urine, glucose, and rat serum samples. Together, these P069-based bioluminescent and fluorescent methods detected *P. aeruginosa* with lower limits of 6.7×10^2 CFU/mL and 1.7×10^2 CFU/mL, respectively [62].

6. *Enterococcus spp.* and *Staphylococcus spp.* Detection

Staphylococcus aureus is a major human pathogen that causes a wide range of diseases and is the leading cause of healthcare-associated infections [63]. *S. aureus* can colonize healthy individuals: approximately 30% of humans are asymptomatic nasal carriers of this bacterium [64], and *S. aureus* carriers have an increased risk of infection and are presumed to be an important source for the spread of *S. aureus* among individuals [65]. Epidemiological data indicate that the population incidence of bacteremia caused by *S. aureus* ranges from 10 to 30 per 100,000 persons per year [66]. This bacterium is known for its ability to become resistant to antibiotics, and this has become an epidemiological problem on a global scale. Diseases induced by methicillin-resistant *S. aureus* strains (MRSA) often occur in epidemic waves initiated by one or a few successful clones; these waves are problematic in both healthcare and community settings [65][67].

Enterococcus spp. lives harmlessly in the digestive tract, but if it spreads to other parts of the human body, it can cause serious health problems. Hospitals are the most common source of these infections. The use of more intensive and invasive medical therapies for humans has caused enterococcal infections to become more common. Increasing antibiotic resistance has also been noted among clinical isolates of enterococci. Many healthcare-associated strains have become

resistant to vancomycin, penicillin, and aminoglycosides. *Enterococcus faecium* is more antibiotic-resistant than *Enterococcus faecalis*; more than half of the pathogenic isolates of the former exhibit resistance to different drugs [68]. Enterococcal infections are often responsible for urinary tract infections among hospitalized patients [69], along with intra-abdominal, pelvic, and soft tissue infections [70], bacteremia [71], and endocarditis [72]. Prompt diagnosis of enterococcal infection is essential for efforts to slow disease progression. One possible strategy is to use the lab-on-chip platform, which offers the benefits of low sample consumption and the possibility for fast and simple analysis [73]. The platform was combined with phage RBPs specific for *Enterococcus* spp. (gp18) and *Staphylococcus* spp. (gp109) for detection of nosocomial pathogens. The utilized proteins had two distinct domains: a C-terminal domain responsible for substrate recognition and receptor binding, and an N-terminal domain engineered to have a 6-His tag [74]. The N-terminal 6-His tag could form a stable complex with heavy metals, such as nickel, and Ni-magnetic beads were used to enable the oriented attachment of the phage RBP [75]. For analysis, bacterial cells were labeled with magnetic nanoparticles functionalized with specific phage proteins, which recognize phage RBP immobilized at the magnetoresistive chip surface. Finally, the magnetically labeled cells were detected by an array of spin-valve sensors on the biochip. This procedure was reported to take less than 2 h and detect both pathogens at concentrations in the range of 10 CFU/mL [76].

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