Analyst

Accepted Manuscript

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Journal Name

ARTICLE

Phage & Phosphatase: A novel phage-based probe for rapid, multi-platform detection of bacteria

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Genetic engineering of bacteriophages allows for the development of rapid, highly specific, and easily manufactured probes for the detection of bacterial pathogens. A challenge for novel probes is the ease of their adoption in real world laboratories. We have engineered the bacteriophage T7, which targets *Escherichia coli*, to carry the alkaline phosphatase gene, phoA. This inclusion results in phoA overexpression following phage infection of *E. coli*. Alkaline phosphatase is commonly used in a wide range of diagnostics, and thus a signal produced by our phage-based probe could be detected using common laboratory equipment. Our work demonstrates the successful: i) modification of T7 phage to carry phoA; ii) overexpression of alkaline phosphatase in E. coli; and iii) detection of this T7-induced alkaline phosphatase activity using commercially available colorimetric and chemilumiscent methods. Furthermore, we demonstrate the application of our phage-based probe to rapidly detect low levels of bacteria and discern the antibiotic resistance of E.coli isolates. Using our bioengineered phage-based probe we were able to detect 10^3 CFU/mL of *E. coli* in 6 hours using a chemiluminescent substrate and 10^4 CFU/mL within 7.5 hours using a colorimetric substrate. We also show the application of this phage-based probe for antibiotic resistance testing. We were able to determine whether an *E. coli* isolate was resistant to ampicillin within 4.5 hours using chemiluminescent substrate and within 6 hours using a colorimetric substrate. This phage-based scheme could be readily adopted in labs without significant capital investments and can be translated to other phage-bacteria pairs for further detection.

Introduction

Pathogenic bacteria represent a significant burden on public and economic health [1-3]. The need for rapid and accurate detection of these pathogens, in both clinical and industrial settings, has spawned a large biosensor industry with a market potential over 500 million USD[4]. The search for new, innovative approaches to detection has driven research into novel probes that exploit molecular interactions, such as bacteriophage, for diagnostic use. A bacteriophage (phage), is a virus which specifically targets bacteria. Phages were first discovered in 1915 and used as antimicrobials, but were quickly supplanted in many areas of the world by the discovery and use of antibiotics[5]. The increasing prevalence of antibiotic resistant bacteria and modern development of molecular tools enabling the specific bioengineering of phages with novel functions has renewed interest in bacteriophages[6, 7]. Phage can be highly specific, they can replicate quickly, and they can be readily propagated. These traits, along with their ability to deliver genetic material to bacterial cells, make them ideal candidates for use as molecular probes for pathogen detection [8].

Phage-based diagnostics have a wide range of applications. In response to foodborne illness, and more recently the passage of the Food Safety Modernization Act, much research has focused on developing phage-based methods for the detection of low concentrations of pathogenic bacteria[8, 9]. The fruits of this research are represented commercially by phage-based diagnostics like VIDAS® UP (Biomérieux, St. Louis, MO) which uses phage components for the detection of *Salmonella*, *E. coli* O157:H7, and *Listeria*, and Sample6 which uses a phage-based bioluminescent reporter for the detection of *Listeria* in environmental samples.

In the area of antibiotic susceptibility testing, rapid DNA-based methods are increasingly supplanting traditional testing methods, but there has been little research into the actual application phage-based detection systems to this area[10-12]. While the detection range of DNA-based multiplex systems has become quite expansive[13], there is a vast reservoir of unknown genes conferring resistance[14] and novel mechanisms of resistance being discovered regularly,[15, 16], making exhaustive testing difficult and possibly resulting in false negatives during testing of clinical isolates. There is also the possibility that genes encoding resistance are present, but not expressed, resulting in a false positive and unnecessary antibiotic treatment. Phage-based antibiotic susceptible testing avoids these issues by focusing on the phenotypic status of the cell, bacterial isolate growth in the presence of the antibiotic, rather than its genotypic status. Phage-based detection also provides several quality controls for testing. Despite the use of proficiency testing, many antibiotic susceptibility labs around the world do not have the appropriate quality control systems in place[17]. Phage host specificity means that potential cross-contamination of an antibiotic susceptible isolate's test by another laboratory isolates of a different species with resistance will not

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

result in a positive signal as it might with a traditional or DNA–based susceptibility test. There is the possibility that a bacterial strain is resistant to the reporter phage used and not the antibiotic, resulting in a false positive. A simple test control that includes growing the isolate in the presence of the phage but without the antibiotic would catch this lack of signal due to phage resistance. These benefits make a strong case the application of phage in novel diagnostics for susceptibility testing.

Challenges for the successful adoption of new diagnostics, particularly in resource limited settings, are their cost, required maintenance, and technician training needed for their operation[18]. We propose a phage-based alkaline phosphatase probe that can be leveraged in a range of detection platforms commonly found in microbiology testing labs (Fig. 1). This scheme would potentially reduce the need for new equipment while allowing for the rapid and sensitive detection of bacteria. Alkaline phosphatase is frequently used as an enzymatic reporter in many diagnostics, its activity can be measured using colorimetric, fluorescent, chemiluminescent, bioluminescent, and electrochemical methods[19-21]. This allows for the potential application of our phage-based platform in widely available commercial diagnostics already optimized for alkaline phosphatase detection.

As a proof-of-principle, we bioengineered the *E. coli* specific T7 phage [22] to carry the *E. coli* gene encoding alkaline phosphatase (*phoA*)[23]. We linked *phoA* to the T7 promoter for overexpression of alkaline phosphatase upon T7 infection of and replication within viable *E. coli*, and demonstrate the detection this T7-mediated alkaline phosphatase activity using commercially available colorimetric and chemilumiscent methods. *E. coli* is commonly used as an indicator of water quality [24], and pathogenic strains of *E. coli* are responsible for urinary tract infections[25], as well as foodborne illnessess[26]. Antibioitc resistant *E. coli* also represent a challenge when treating infections in clincal settings[27], thus novel methods that improve time-to-detection of *E. coli* are of interest. While others have used *E. coli* specific phage for either colorimetric[28] or bioluminescent [29] detection, to our knowledge we are the first to have successfully inserted a gene for alkaline phosphatase[7, 8, 30]. We demonstrate potential applications of our phage-based probe to: i) enable the detection of low concentrations of *E. coli* cells, (10^3-10^4 CFU/mL) in 6-8 hours in broth; and ii) improve time to result for antibiotic susceptibility testing of bacterial isolates. Future work will be necessary to demonstrate the robustness of this concept in more complex sample matrices, but we believe this scheme can be readily reproduced using other phage-bacteria combinations, and could be easily adopt in many laboratories without the need for added equipment or media.

Materials and Methods

Bacterial strains, bacteriophage strains, media culture, and enumeration.

The following bacterial and bacteriophage strains were purchased from EMD Millipore (Billerica, Massachusetts): *E .coli* BL21, *E. coli* BLT5403, and bacteriophage T7Select® 415-1. Overnight cultures of both *E. coli* strains used in this study were grown at 37 °C with 200 rpm shaking in 35 mL of Luria Broth (LB), pH 7.5, contained in a 150 mL Erlenmeyer flask. Before use overnight cultures were serially diluted and plated on LB agar to confirm bacterial concentration for subsequent experiments. A double agar overlay plaque assay on LB[31] was used to enumerate phage samples. The only modification to above procures was the inclusion of 100 µg/mL of ampicillin to broth and agar for the growth of *E. coli* BLT5403 to maintain its plasmid for resistance [32].

Construction and isolation of engineered bacteriophage

In order to modify a T7 phage that carries the *E. coli* gene for alkaline phosphatase (*phoA)* we designed a specific construct, 1743 base pairs in size, to enable T7-induced overexpression of *phoA* in *E. coli* within a pUC57 plasmid by GenScript USA Inc. (Piscataway, NJ). Our *phoA* construct was amplified with standard M13 forward and reverse primers using the iProof high-fidelity PCR kit (Bio-Rad Laboratories, Hercules, CA). All PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Valencia, CA), and digested by EcoRI and HindIII. These restriction enzymes were purchased from New England Biolabs (Ipswich, MA). Our digested construct was then ligated into the T7 Select® 415-1 genome vector arms using T4 DNA ligase (Promega, Madison, WI) and packaged using the T7 Select® packing kits (EMD Millipore) to create $T7_{phoA}$ (Fig. 2). We used the T7 Select® kit's packaging control DNA, which contains the S•Tag™, as a control phage, $T7_{control}$ (Fig. 2), which could not induce alkaline phosphatase overexpression. Following packing, $T7_{phoA}$ and $T7_{control}$ were propagated and plated as outlined by the T7 Select® kit's protocol. Individual plaques were selected, dipped into 100 µL of LB, and stored at 4 °C. All isolated plaques were PCR screened for the appropriate size insert with the T7Select® Up and Down primers using the iProof PCR kit. Plaques containing the appropriate sized insert were propagated on *E. coli* BL21, and the resulting lysates re-screened to confirm the presence of our *phoA* insert. These lysates were passed through a 0.22 µm SCFA filter (Corning Life Science, Corning, NY), tittered, and stored at 4 °C and used as our $T7_{phoA}$ and $T7_{control}$ phage stock for the further experiments.

Colorimetric detection of alkaline phosphatase activity

Colorimetric detection of alkaline phosphatase activity was performed using the SensoLyte® pNPP Alkaline Phosphatase Assay Kit (AnaSpec, Inc., San Jose, CA). The sample (50 µL) was mixed with 50 µL of the kit's p-Nitrophenyl Phosphate (pNPP) colorimetric alkaline phosphatase substrate in a clear 96-well plate, incubated at 37 °C, and absorbance at 405nm read every 10 minutes, over 90 minutes, using the Synergy 2 plate reader (BioTek Instruments, Inc., Winooski, VT). Blanks consisted of LB and pNPP substrate.

Chemiluminescent detection of alkaline phosphatase activity

Chemiluminescent detection was performed with two substrates. The first was alkaline phosphatase substrate Lumigen APS-5 (Lumigen, Inc., Southfield, MI). 50 µL of sample was mixed with 50 µL of APS-5 in a black 96-well plate at room temperature. Blanks consisted of LB and substrate. The plates were immediately read using a Synergy2 plate reader (BioTek Instruments, Inc., Winooski, VT) with Ex/Em filters Plug/460 nm.

The second method of chemiluminescent detection was performed using components of the Phospha-Light™ SEAP Reporter Gene Assay Kit (Thermo Fisher Scientific Inc., Waltham, MA). A 50 µL aliquot of a 1:20 dilution of the kit's CSPD® chemiluminescent substrate in the kit's reaction buffer, which contains the Emerald™ chemiluminescence enhancer, was mixed with 50 µL of sample in a black 96-well plate at room temperature. Blanks consisted of LB and substrate. Plates were place in the Synergy2 plate reader (BioTek Instruments, Inc., Winooski, VT) for 10 minutes and read with Ex/Em filters set to Plug/Hole, respectively.

Confirming alkaline phosphatase overexpression

Six 150 mL Erlenmeyer flasks containing 35 mL of LB were each inoculated with a 150 µL aliquot of an overnight culture of BL21. These flasks were then incubated at 37 °C for 3 hours with 200 rpm shaking, and the cultures confirmed to have reached and $O.D._{600}$ > 0.6. Three cultures were then inoculated with 15 µL of $T7_{phoA}$ phage stock, and the remaining three inoculated with 15 µL of $T7_{control}$ phage stock. The cultures were incubated for 2 hours under the same conditions. The resulting lysates were then transferred to a 50 mL conical tube tubes and spun at 7598 × g on a Fiberlite F21-8x50y fixed angle rotor (Thermo Fisher Scientific Inc., Waltham, MA) for 10 minutes at room temperature. The supernatant of each was filtered through a 0.22 µm SCFA filter (Corning Life Science, Corning, NY) and stored individually at 4 °C. All lysates were then tested for alkaline phosphates activity using both pNPP and the APS-5 substrates.

Bacterial detection using $T7_{phoA}$

Four separate overnight cultures of *E. coli* BL21 were serially diluted in LB to achieve 10^5, 10^4, 10^3 and 10^2 CFU/mL. 900 µL of each dilution and a negative control of LB were placed in 15 × 100 mm test tubes, and mixed with 100 µL of 10^3 PFU/mL of $T7_{phoA}$ in LB. Each combination, for each overnight culture, was performed in triplicate. The samples were incubated at 37 °C with 200 rpm shaking for 6 hours. Samples were then passed through a 0.22 µm SCFA filter, stored at 4 °C, and alkaline phosphatase activity was determined using all three substrates.

Determining antibiotic susceptibility of bacterial isolates using $T7_{phoA}$

E. coli BL21 and BLT5403 were streaked onto the appropriate plates for single colonies. A colony of each was selected using a sterile loop and inoculated into a test tube containing 900 µL of either LB or LB containing 100 µg/mL of ampicillin. An un-inoculated test tube of LB was used as a negative control. All colony-antibiotics treatments were performed in triplicate. The tubes were then incubated at 37 °C with 250 rpm shaking for 30 minutes. Then 100 µL of 10^3 PFU/mL of $T7_{phoA}$ in LB was added to each tube. The tubes were incubated under the same conditions for 3.5 hours. For chemiluminescent detection a 50 µL samples of each tube was taken and mixed with 50 µL of the Phospha-Light™ substrate solution in a black 96-well plate and read as previously described. For colorimetric detection a 50 µL samples was mixed with 50 µL of the pNPP substrate and incubated for 1.5 hours at 37 °C in a clear 96-well plate. The plate was then read for absorbance at 405 nm using the Synergy 2 reader.

Statistical Analysis

Data was evaluated for statistically significance in Origin Pro version 9.0.0 (Northampton, MA). For both experiments looking at alkaline phosphatase overexpression versus a control, an unpaired one-sided t-test, assuming unknown and unequal variances, with an alpha level of 0.05 was used to test for significance. This includes the initial phosphatase expression experiment, where the average signal-to-noise ratio of all three $T7_{phoA}$ lysates was compared against that of $T7_{control}$. This was also done for the limit of detection experiment where the signal-to-noise ratio of each bacterial concentration level was compared to that of the control containing no cells. For the antibiotic resistance experiment the signal-to-noise ratio was compared between the two treatments (with/without antibiotic) within each strain type using an unpaired two-sided t-test assuming unknown and unequal variances, with an alpha level of 0.05. In all figures error bars represent one standard deviation (SD) ± from the mean and a star (*) indicates a significant difference ($p < 0.05$) between the compared sets of data.

Results and Discussion

Phage Construction

To construct our alkaline phosphatase reporter phage, we designed a genetic construct carrying *phoA* (Fig. 2a) for insertion in the T7 genome (Fig. 2b). T7 is a well-studied phage that broadly targets *E. coli* [22], and is used for phage-display [33]. Our genetic construct contains multiple components. Beginning from the 5'-terminus, we included the 1.3s biotin subunit from *Propionibacterium shermanii* transcarbonylase. The T7 Select® cloning kits enables phage-display, allowing us to fuse the 1.3s subunit to 10B capsid protein of T7[32]. The inclusion of this "biotin-tail" sequence has been shown to allow *in vivo* biotinylation of the fusion protein by *E. coli* [34]. At the 3' end the 1.3s gene we incorporated a stop codon so that our alkaline phosphatase gene would not also be fused to the capsid, but rather free in solution. To enable T7 mediated expression of alkaline phosphatase we incorporated the T7 promoter and ribosome binding site sequences from the pET-3a plasmid (EMD Millipore). Our synthetic *phoA* sequence was initially based on the *E. coli* sequence described by [23]. This *phoA* gene is flanked on the 5' by a His-tag/TEV cleavage site, and on the 3' end by a 5× arginine tag. Finally we incorporated three restriction sites into our construct, EcoRI at the 5' terminus, and SalI followed by HindIII at the 3' terminus. The *phoA* coding sequence of the synthetic construct was codon optimized for expression within *E. coli* based on their proprietary algorithms. The full sequence of the construct can be found in the supplemental materials. We inserted our construct into T7 using the T7 Select®415-1 kit (Fig. 2c) and created a T7 control using the kit's packing control DNA (Fig. 2d). PCR was used to screen and isolate phage plaques carrying our *phoA* construct and the control.

Confirming ALP overexpression

The alkaline phosphatase gene *phoA* is endemic to *E. coli*[23], and is typically expressed at low levels unless the cells are starved for phosphate[35]. To confirm that $T7_{phoA}$ exhibits increased alkaline phosphatase production over background, and not due to the stress of the phage infection itself, we compared alkaline phosphatase activity in cells infected and lysed by our $T7_{phoA}$ and our $T7_{control}$. We added an aliquot of an overnight *E.coli* BL21 culture to fresh LB and incubated it for 3 hours to ensure the cells achieved logarithmic growth. We then inoculated the cultures with either $T7_{phoA}$ or $T7_{contorl}$, allowing the phage infection and replication cycle to occur two hours before testing the resulting lysates for phosphatase activity. We tested for activity using both colorimetric pNPP (Fig. 3a) and Lumigen-APS 5 chemiluminescent substrate (Fig. 3b). There was a 10-fold signal-noise-ratio or greater difference in alkaline phosphatase activity between our control phage and our $T7_{phoA}$, suggesting that infection of *E. coli* by our $T7_{phoA}$ does result in alkaline phosphatase overexpression. Other research in our lab has demonstrated the speificity of T7 phage-based detetion of *E. coli* in the presence of competitive bacterial species such as *S. enterica*, *S. aureus*, *P. aeruginosa*[36].

Bacterial cell limit of detection using T7*phoA*

We incubated several concentrations of *E. coli* BL21, 10^5 to 10^2 CFU/mL, and a negative control of LB, with 10^2 PFU of $T7_{phoA}$, for 6 hours at 37 °C. We then filtered the samples to remove cells and tested for alkaline

phosphatase activity using three substrates: pNPP, the Phospha-Light™, and Lumigen APS-5. Significant alkaline phosphatase activity from initial bacterial levels of 10^4 CFU/mL and greater were detected using the colorimetric and chemiluminescent substrates (Fig. 4). It should be noted that the pNPP colorimetric assay required a 90 minute reaction time for the signal, resulting in a total assay time of 7.5 hours. For comparison, the Phospha-Light™ method only required 10 minute incubation before testing. The Lumigen APS-5 was read immediately following addition of the substrate, so does not significantly increase assay time. Furthermore, with the Lumigen substrate we were able to detect an initial cell concentration of 10^3 CFU/mL (Fig. 4c).

The limit of detection (L.O.D.) of our scheme is driven by two factors. The first is the senstivity of the method used to detect alkaline phosphatase activity, as illustrated by the difference in L.O.D. of the colormetirc pNPP and the chemiluminescent Lumigen APS-5. The second factor is the total number of cells infected, which impacts the total amount of alklaine phosphatase produced. There is the potential to incorporate more sensitive detection methods for alklaine phosphatase to improve our limit of detection, for example the use of an electrochemical redoc-cycling scheme for alkaline phosphatase been shown to improve sensitivity[37]. There is also the potential to incorporate a pre-enrichment step, commonly used in when testing food samples[38], to increase bacterial levels prior to introduction of our T7$_{phoA}$. These options represent future areas of research to improve the sensitivity of this phage-based scheme.

Determining antibiotic susceptibility of *E. coli* isolates

Traditional tests for determining the antibiotic susceptibility of a bacterial isolate do so by looking for bacterial growth in the presence of the antibiotic[39]. Broth microdilution Minimal Inhibitory Concentration (MIC) susceptibility testing in 96-well plates is a widely adopted method in clincal laboratories for assesing an isolates antibiotic susceptibility[39]. The baterial inoculum is typically prepared from isolated colonies culutred on a non-selective agar plate for 18-24 hours[39]. Several colonies, 3 to 5, are re-suspended in broth, standardized to a specific density, and then used innoculate wells containing varying concentrations of the antibiotic of interest[39]. The plates are then incubated for 16-24 hours, depending on the baterial species, and anaylzed for growth[39]. We were interested in determining if our phage-based probe had the potential to improve detection time of this later incubation step. If a bacterial isolate were resistant to a given antibiotic it should grow in broth with and without antibiotic. If we add T7$_{phoA}$, we should see phage infection, phage replication, and the production of our alkaline phosphatase reporter in both treatments. If the bacterial isolate were sensitive to a given antibiotic there will be bacterial death in the broth containing the antibiotic, resulting in no phage replication, and no alkaline phosphatase production. We should see therefore see a difference in alkaline phosphatase signal between the treatments.

For a proof-of-principle application of our modified phage, we selected colonies of *E. coli* BL21, which is susceptible to ampicillin, and colonies of BLT5403, which carries a plasmid conferring resistance to ampicillin, and used them to inoculate either broth containing 100 µg/mL of ampicillin or broth not containing the antibiotic and incubated for them for 30 minutes. T7$_{phoA}$ was then added and the samples were incubated for an additional 3.5 hours. The samples were then analyzed for alkaline phosphatase activity using pNPP and Phospha-Light™ substrates (Fig . 5). In our initial run, we had an overflow in pNPP absorbance readings for three samples - one of the BLT5402 colonies exposed to ampicillin and two without - so for analysis we set the absorbance reading for those sample to be equivalent to the max read 405 nm absorbance for the run, which was 3.82. We repeated the pNPP portion of the trial, the results are an average these two trials (Fig 5b). For both substrates, there were no significant differences in signal between antibiotic treatments for *E. coli* strain BLT5403 strain, indicating that the strain was resistant to ampicillin. There were a significant differences in signal between the treatments for *E. coli* strain BL21, indicating that the strain was sensitive to ampicillin. Total assay time was < 4.2 hours using the chemiluminescent substrate, and < 6.5 hours using pNPP. These experiments suggest there incorporation of our phage-based assay in the second incubation step of the MIC susceptibility test could reduce the 16-24 hours delay for results[39]. While the colorimetric assay takes longer than the chemiluminescent one, for resource limited labs the pNPP assay does provide the ability for visual interpretation of results as can be seen in Fig. 3c, which is a picture of the 96-well plate from the first pNPP trial.

The increasing level of antimicrobial resistance among clinical and foodborne bacteria is a major public health concern driving the need for rapid diagnostics[10]. The application of phage for the rapid detection of antimicrobial resistance is still in its infancy. MicroPhage Inc. (Longmont, CO) had developed an FDA approved phage-based lateral flow device for the rapid detection of methicillin resistant (MRSA) *S. aureus*[40], though it is no longer commercial available[41]. One of the challenges with lateral flow devices like that of MicroPhage's, is that format does not lend itself to screening of a large number of samples. Our T7$_{phoA}$ platform is readily leverage into 96-well microtiter plates, which are commonly used in for antibiotic testing[39]. This format allows for the simultaneous analysis of a large number of samples and enables automation. We believe this platform could be readily translated to phages with specificity to other bacteria which have public health relevance, as the bioengineering of phage has been readily be demonstrated[30]. Future research standardizing our bacterial inoculum procedure and demonstrating the robustness of our phage-mediate alkaline phosphatase-based platform for antibiotic resistance testing will be needed.

Future applications: food and water testing

Alkaline phosphatases are ubiquitously found among bateria, animal, and plants[42] which introduces background noise for our phage-based scheme when applied to food and water samples. There a several methods which can be used to reduce this potential interferance. Bacterial alkaline phosphatases are more heat stable than those of animal and plant origin, and researchers have shown the effective use of heat treatment step to differeniate bacterial alkaline phosphatases from those of both plant[43] and animal origin[44] when testing foods and could be implemented as part of our assay.

Furthermore, we have designed several elements into T7$_{phoA}$ to address background noise in future experiments as we explore the applications of our proof-of-concept in real world testing. Our synthetic alkaline phosphatase contains both an N-terminus His-tag and C-terminus arginine tag (Fig. 2), both of which provide ways to selectively capture our reporter enzyme, and reduce background interference from endemic phosphatases in a sample. Our phage-based construct is also designed to enable bacterial separation. We have incorporated a biotin tag fused to the capsid protein

(Fig. 2), which allows us to attach our phage to magnetic beads coated with streptavidin, and then use these phage-magnetic beads for selective separation of *E.coli* cells from a sample. Researchers have demonstrated the parity of phage-magnetic beads to immunomagnetic separation techniques[45], and the succesfull application of these beads to selective separate a bacterial from water samples[36]. These selective elements, on the both the enzyme and phage, can potentially be used solely or in tandem to help overcome a wide range of challenges from enzymatic inhibitors to endemic phosphatases to visual inteference of signal that are common when attempting to test food and water samples.

Conclusion

Phage-based diagnostics offer powerful platforms for the rapid detection of bacteria. There has been an emphasis on using phage to detect low numbers of bacteria, but there are broader applications for phage-based diagnostics. In applications where initial bacterial loads are high, like antibiotic testing or post-primary enrichment, phage-based detection can be quite rapid and specific. In these application, phage-based diagnostic are also attractive in comparison to PCR or immunological based methods as phage are cheaply produced, specific, and as we demonstrated, readily engineered to leverage a wide range of easy-to-use, commercially available detection platforms. Another advantage to our scheme is that unlike immunological[46] and PCR-based[47] methods, phage-based detection can distinguish between viable and non-viable cells, thus lowering the incidence of false positives. More research bioengineering flexible, phage-based reporters and demonstrating multiple forms of their applications is needed to illuminate the potential of phage-based detection and ensure its successful adoption in real world diagnostics to improve public health.

Acknowledgements

The authors would like to acknowledge the USDA NIFA National Needs Fellowship (2011-38420-200044), USDA NIFA Pre-Doctoral Award (2015-67011-22805), the Center for Hierarchical Manufacturing, National Science Foundation, CMMI-1025020, and the Northeast Alliance for Graduate Education and the Professoriate and NIH IMSD grant GM099649. for their support.

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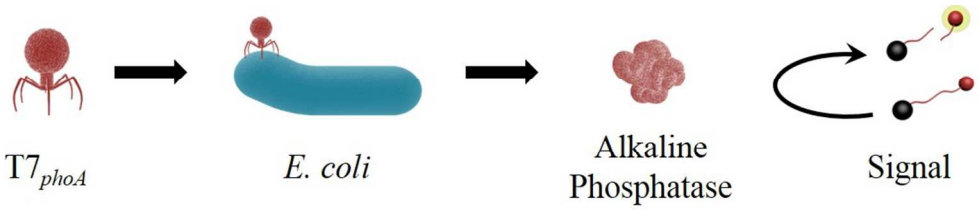
Fig. 1 Phage-based probe. $T7_{phoA}$ is added to a sample. If viable E. coli are present within the sample, phage infection, replication, and alkaline phosphatase overexpression occurs. The alkaline phosphatase reporter can then be detected being using a variety of substrates.

Fig. 2 Diagram of DNA Constructs. (A) Our construct containing *phoA*. (B) Genome of T7 Select® 415-1 indicating 10B capsid protein and insertion site. (C) Genome of $T7_{phoA}$. (D) Genome of $T7_{control}$ with S•Tag™.

Fig.3 Confirming alkaline phosphatase expression. Samples A1-3 are lysates form $T7_{phoA}$, samples C1-3 are lysates from $T7_{control}$. a) alkaline phosphatase signal with pNPP substrate, b) alkaline phosphatase signal with Lumigen APS-5 chemiluminescent substrate.

Fig.4 Limit of detection. Signal to Noise Ratio of alkaline phosphatase activity after 6 hour incubation of initial concentrations of *E. coli* with 10^2 PFU/mL of $T7_{phoA}$. Substrates: a) pNPP; b) PhosphaLight™; c) Lumigen APS-5.

Fig.5 Detect antibiotic resistance. Signal to Noise Ratio of alkaline phosphatase activity from *E. coli* stains incubated with 10^2 PFU/mL of $T7_{phoA}$ in LB with and without ampicillin. Substrates: a) PhosphaLight™; b) pNPP. c) Visual of wells with pNPP substrate.



Phage-based probe. $T7_{phoA}$ is added to a sample. If viable *E. coli* are present within the sample, phage infection, replication, and alkaline phosphatase overexpression occurs. The alkaline phosphatase reporter can then be detected being using a variety of substrates.
193x42mm (150 x 150 DPI)

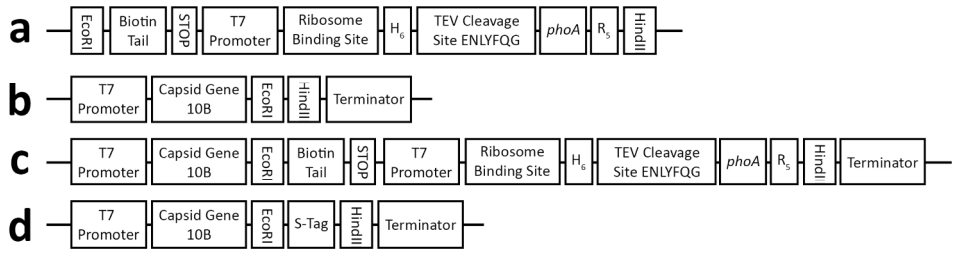
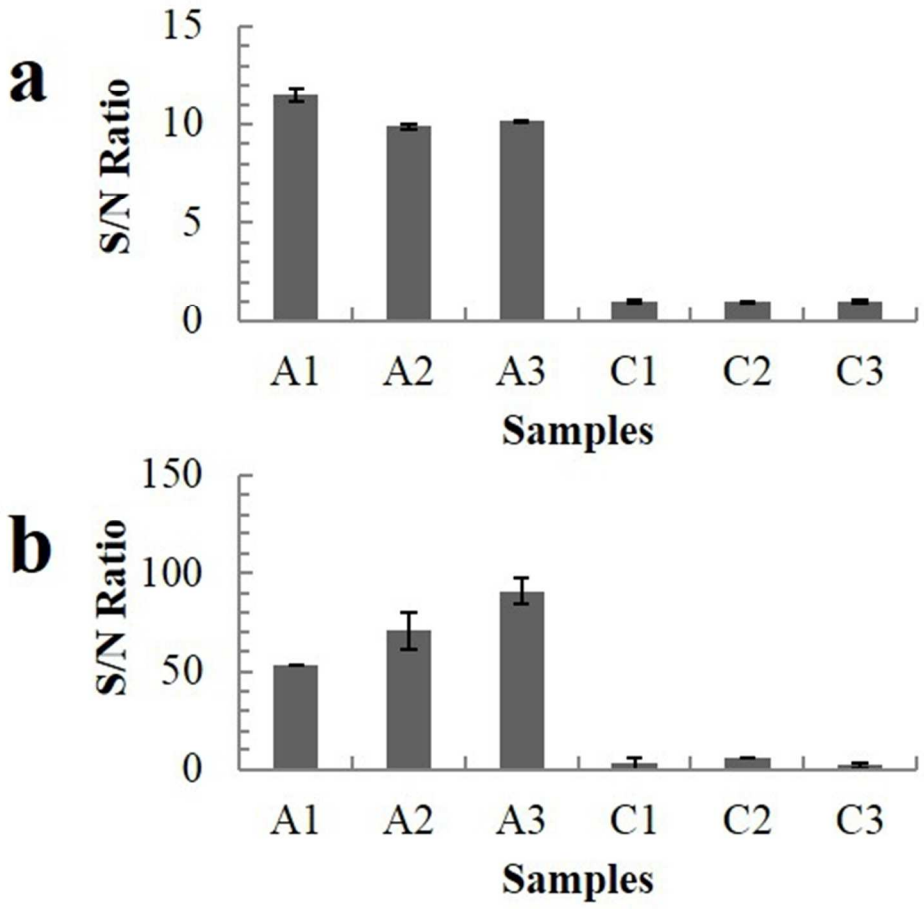
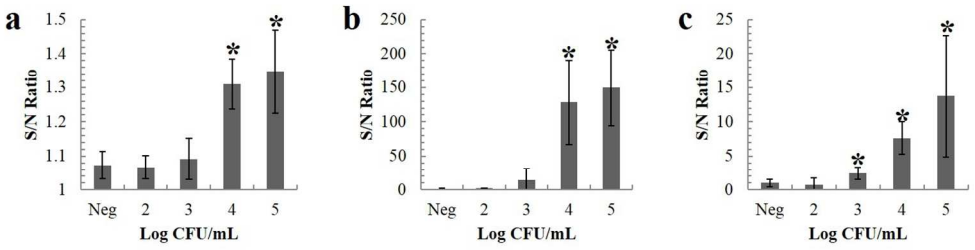


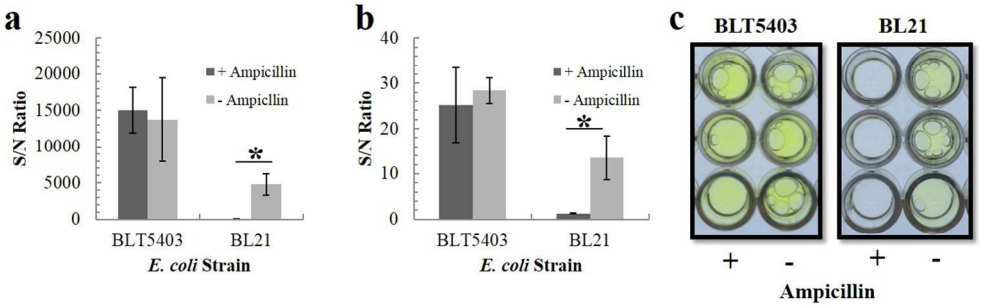
Diagram of DNA Constructs. (A) Our construct containing phoA. (B) Genome of T7 Select® 415-1 indicating 10B capsid protein and insertion site. (C) Genome of $T7_{phoA}$. (D) Genome of $T7_{control}$ with S•TagTM.



Confirming alkaline phosphatase expression. Samples A1-3 are lysates form $T7_{phoA}$, samples C1-3 are lysates from $T7_{control}$. a) alkaline phosphatase signal with pNPP substrate, b) alkaline phosphatase signal with Lumigen APS-5 chemiluminescent substrate.
98x95mm (150 x 150 DPI)



Limit of detection. Signal to Noise Ratio of alkaline phosphatase activity after 6 hour incubation of initial concentrations of E. coli with 10^2 PFU/mL of $T7_{phoA}$. Substrates: a) pNPP; b) PhosphaLight™; c) Lumigen APS-5.
245x68mm (150 x 150 DPI)



Detection of antibiotic resistance. Signal to Noise Ratio of alkaline phosphatase activity from *E. coli* stains incubated with 10^2 PFU/mL of $T7_{phoA}$ in LB with and without ampicillin. Substrates: a) PhosphaLight™; b) pNPP. c) Visual of wells with pNPP substrate. A star (*) indicates a significant difference ($p < 0.05$) in signal-to-noise ratio between the two treatments (with/without antibiotic)within each strain type.
230x77mm (150 x 150 DPI)