Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

Phage based electrochemical detection of *Escherichia coli* in drinking water using affinity reporter probes

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1. Materials and methods

1.1 Construction of engineered phages

In this work, a highly active reporter enzyme was fused to an affinity binding motif and subsequently inserted into the genome of a phage using standard cloning approaches as described previously [1]. Specifically, five tandem repeats of the gold binding peptide (MHGKTQATSGTIQS) were fused to the N-terminus of an alkaline phosphatase allele possessing two mutations that confer a significant increase in enzymatic activity (D153G/D330N) [2]. This novel genetic fusion was synthesized as a linear DNA fragment (gBlock) within an expression construct complete with T7 regulatory sequences expression and flanked by homology to T7 for insertion via homologous recombination. T7 Genomic DNA was purified and digested with *Hind*III to serve as a cloning vector. The reporter gene insert was added to the genomic DNA at a 2:1 molar ratio and assembled with NEBuilder® Hifi DNA Assembly Master Mix (NEB, Ipswitch, MA). The assembled DNA was transformed in electrocompetent *E. coli* DH10B cells (MegaX, ThermoFisher) under standard conditions. Full genome sequencing confirmed the presence of the insert with no other insertions, mutations or deletions within the insertion site. T7 phages carrying a reporter gene for ALP were designated NRGp1 (Accession: MH651795), while T7 carrying a GBP-ALP reporter was designated NRGp7 (Accession: MH703728).

E. coli S2157 cells with plasmid pSB2991 encoding ALP and plasmid pSB3057 encoding five repeat tandem gold binding peptides fused to ALP were provided by Dr. Stanley Brown (University of Copenhagen, Denmark)

1.2 Optimization of the binding condition of GBPs-ALP on gold electrodes

An aliquot of 100 μL GBPs-ALP in LB expressed from the NRGp7 phages were deposited on the screen-printed gold electrodes (SPGEs) (Dropsens, Asturias, Spain). Then the electrodes were placed at room temperature (22 ℃) and 37 ℃ for 10, 30, 60, 90 and 120 minutes, respectively, allowing the immobilization of ALP on the surface of gold electrodes through the function of GBPs at different conditions. Then gold electrodes were washed three time with 0.05M tris buffer (pH 10.0) and dried with nitrogen gas. Because the optimal pH for ALP was reported as pH 10.0. Substrate solution containing 1mM AAP and 1 mM $AgNO_3$ (100 μL) were placed on the gold electrode and reacted with the ALP immobilized on the electrode at 37 ℃ for 30 minutes. Following by the enzymatic reaction, the electrodes were rinsed with water and a 0.6M potassium nitrate (KNO_3) solution (60 μL) was dropped on the electrode. LSV was performed to measure the peak current for the samples with different binding.

2. Results and discussion

2.1 Performance of GBPs-ALP expressed from E. coli and construction of NRGp7 phages

Gold binding peptide, encoded by a 14-amino acid sequence (MHGKTQATSGTIQS), was first selected using cell surface display [3]. This sequence was selected because it has shown high affinity towards gold surfaces. Interestingly, the peptide lacks cysteine which is generally used to form thiol linkage to gold in this molecular linker. The binding mechanism of this gold binding peptide is based on multiple noncovalent interactions. Molecular level details such as specific recognition and binding affinity are poorly understood and being investigated [4, 5]. The gold binding peptide has been used in several studies such as a fusion partner for the immobilization on gold surface [6] or the modification of gold nanoparticles [7]. The work of Kacar et al [8] studied the property of this binding peptide fused ALP expressed from *E. coli* and demonstrated that five repeats of GBP displayed the best bi-functional property. We also investigated the effectiveness of

GBPs-ALP from *E. coli* S2157 cells with plasmid pSB3057 encoding five repeat tandem gold binding peptides fused to ALP in our electrochemical system (Fig. S1). Additionally, the enzyme ALP used here had two mutants with higher enzymatic activity. Hence, five tandem repeats of GBP were genetically fused to N-terminus of ALP and the gene expressing GBPs-ALP was inserted into the genome of T7 phages.

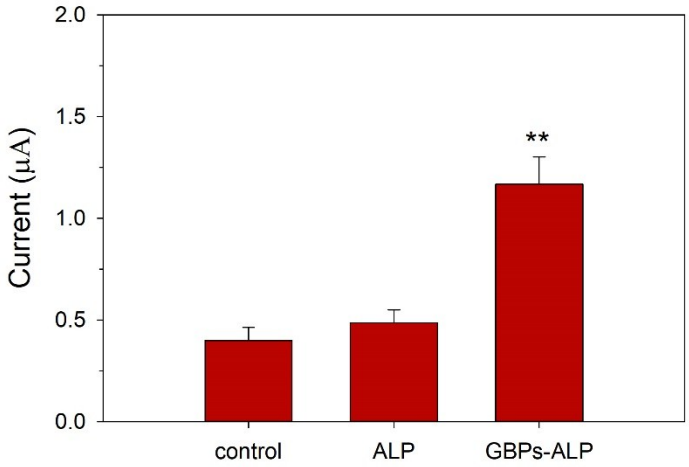

Figure S1. Electrochemical signal obtained using fusion protein GBPs-ALP expressed from *E. coli* with plasmid pSB3057, single enzyme ALP expressed from *E. coli* with plasmid pSB2991, and control (LB broth), respectively. One asterisk (*) represents a significant difference ($0.01 < p < 0.05$) and two asterisks (**) represents a significant difference ($p < 0.01$) between each treated group with the control group. The error bars represent the standard deviation of three replicates.

2.2 Specificity of NRGp7 phages

The specificity of this proposed method was tested using four different bacterial strains including *E. coli* (ECOR-13), *Salmonella enterica* (*S. enterica*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and a mixture of all strains. A phage can specifically infect a subset of bacteria strains using its tail fiber. A sample without bacteria was performed as a negative control. The concentration of each bacteria used in this investigation was 10^6 CFU/mL. Figure S2 showed the electrochemical detection results after 2 h of incubation. It can be observed that a significant signal was obtained only when the sample had *E. coli*. The other samples without *E. coli* obtained the similar signal as the negative control. The results demonstrated the NRGp7 phages can only specifically recognize and infect *E. coli* and this proposed method showed good specificity to *E. coli*.

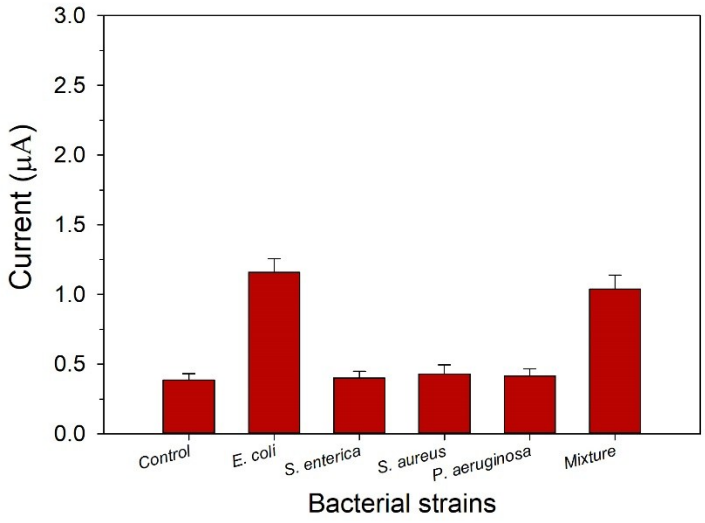

Figure S2. Electrochemical signal obtained for the detection of 10^6 CFU/mL of *E. coli*, *S. enterica*, *S. aureus*, *P. aeruginosa*, and a mixture of all these strains, respectively. A sample without bacteria was used as a negative control. The error bars represent the standard deviation of three replicates.

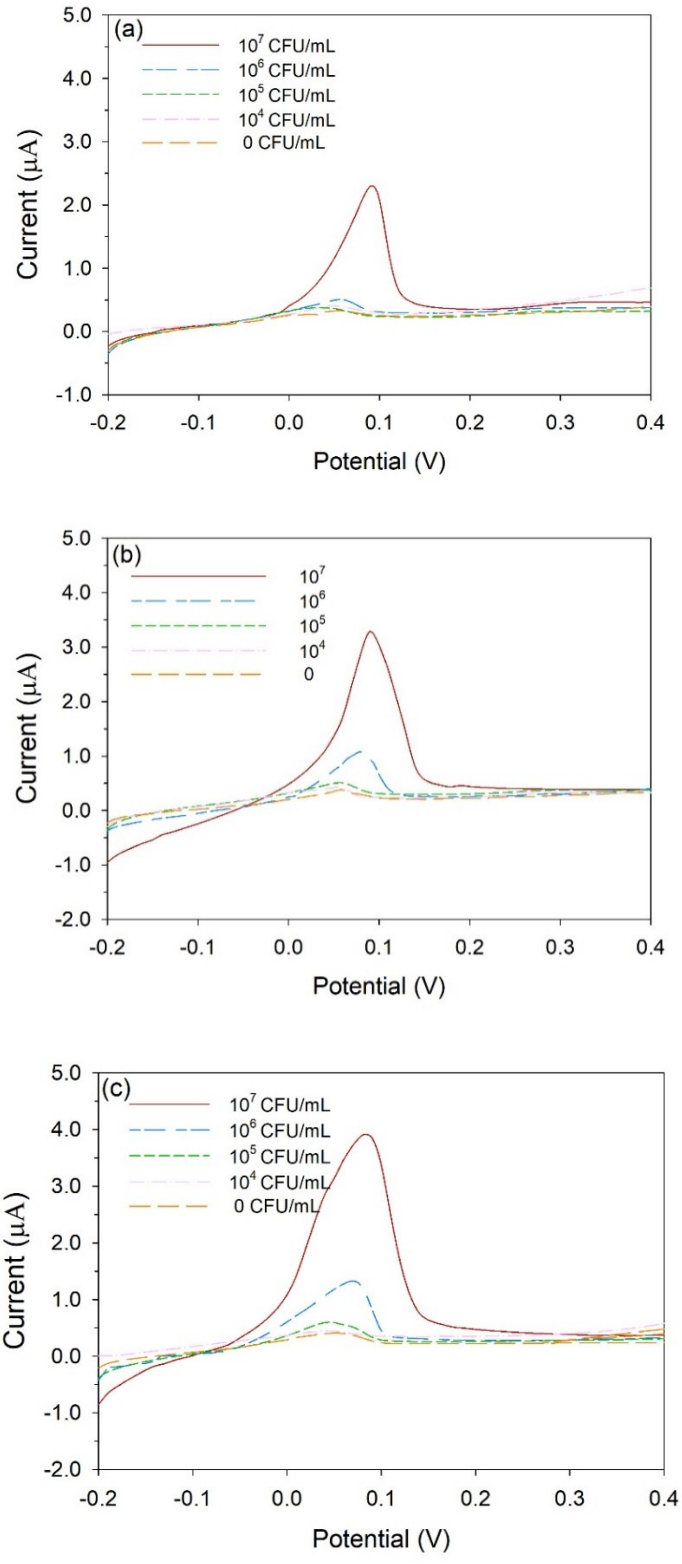

Figure S3. LSV curves for increasing concentration of *E. coli* (0, 10^4, 10^5, 10^6, and 10^7 CFU/mL) after (a) 1hour, (b) 2 hours, and (c) 3 hours of incubation for engineered phages and *E. coli*.

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