Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

A Sandwich-type Bacteriophage-Based Amperometric Biosensor for the Detection of Shiga Toxin-Producing *Escherichia coli* Serogroups in Complex Matrices

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Supplementary figures and tables.

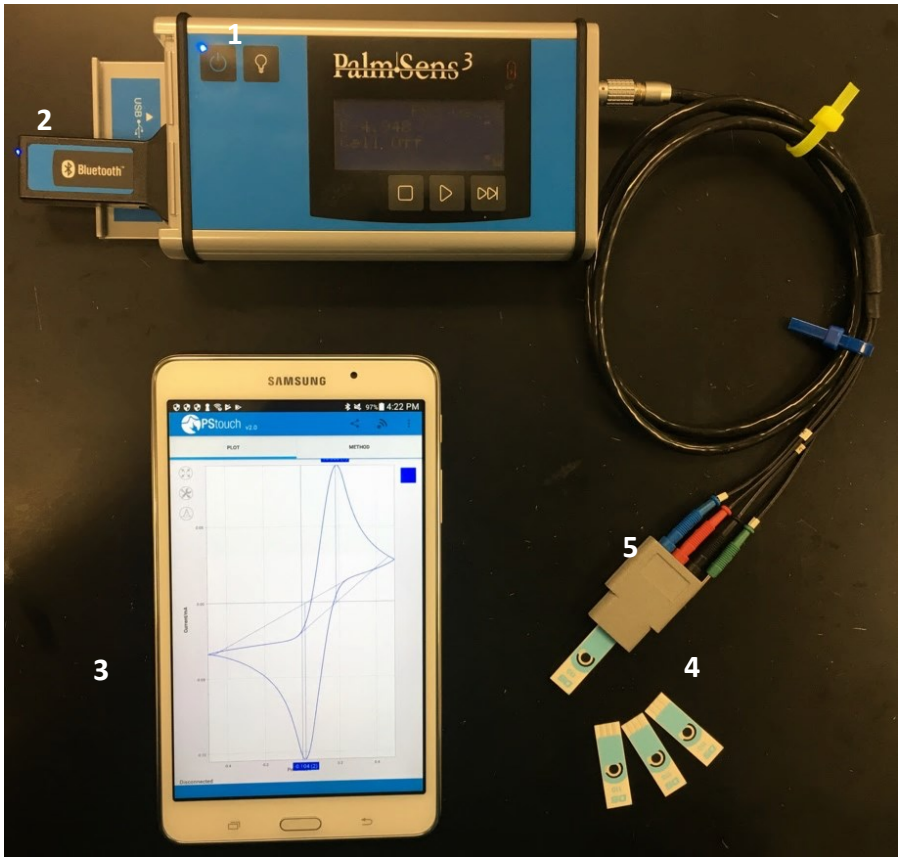

Fig. S1. The complete configuration of the biosensor architecture. 1-PalmSens3, 2-Bluetooth dongle, 3-Android device, 4-SPCEs, 5-SPCE holder, and connector.

Table S1. List of natural environmental water samples from Pescadero, CA. All samples were provided by USDA-WRRC-ARS (PSM Unit).

Sample/Site	Sample ID	Description	Sampling Sites
1	P1-D	Sediment water samples	Pescadero, CA
2	P8-W	Irrigation water samples	Pescadero, CA
3	P1-W	Irrigation water samples	Pescadero, CA
4	P7-W	Irrigation water samples	Pescadero, CA

Table S2. Primers for *stx* genes. [1]

Name	Sequence
*stx*1 For(*stx*1-1-F)	5' - CATCGCGAGTTGCCAGAATG - 3'
*stx*1 Rev(*stx*1-1-R)	5'- AATTGCCCCCAGAGTGGATG - 3'
*stx*2 For(*stx*2-5-F)	5' - GTATAC GATGACGCCGGGAG - 3'
*stx*2 For(*stx*2-5-R)	5'- TTCTCCCCACTCTGACACCA - 3'

The conventional PCR conditions were as follows, denaturation at 95 ^{o}C for 2 min; 35 cycles of 30 sec denaturation at 95 ^{o}C, annealing at 56 ^{o}C for 30 sec, elongation at 72 ^{o}C for 30 sec and a final extension at 72 ^{o}C for 5 min. The initial standard plate count method using MacConkey Sorbitol agar was conducted for STEC isolation and quantification. All plates were incubated overnight at 37 ^{o}C.

Table S3. **Standard curve of the high-titer representative bacteriophage stocks (PFU mL^{-1}) and its concentration in μg mL^{-1} .**

Titer Level (PFU mL^{-1})	Concentration (μg mL^{-1})
10^9	550
10^{10}	720
2.5×10^{10}	900
7×10^{10}	950

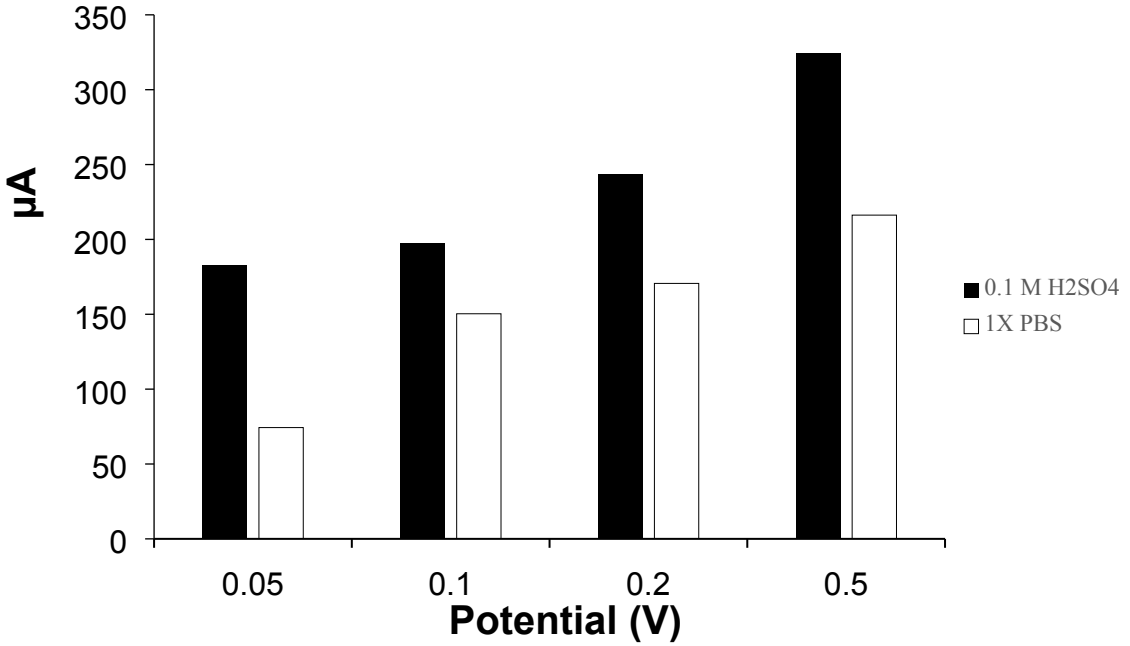

Figure S2. Bar graph representation of the electrochemical characterization of unmodified SPCEs. Voltammograms were recorded at increasing scan rates of 0.5 mM $K_3[Fe(CN_6)]$ in two different supporting electrolytes. The peak separation (ΔE_p, mV) between the oxidation and reduction potentials were recorded as a function of the surface of SPCEs.

Table S4. Modification of SPCE. Characterization of SPCE by measuring ΔE_p (mV) after modifying it with various reagents.

Modified SPCE	ΔE_p (mV)
CMD-dextran modified	160.481
EDC-NHS modified	201.023
Streptavidin modified	216.228
Bacteriophage modified	236.499
FeDC modified	216.227
AuNP modified	207.781
BSA modified	216.227
Casein modified	206.091
Protein free modified	206.091
PEG	195.956

(A) **(B)**

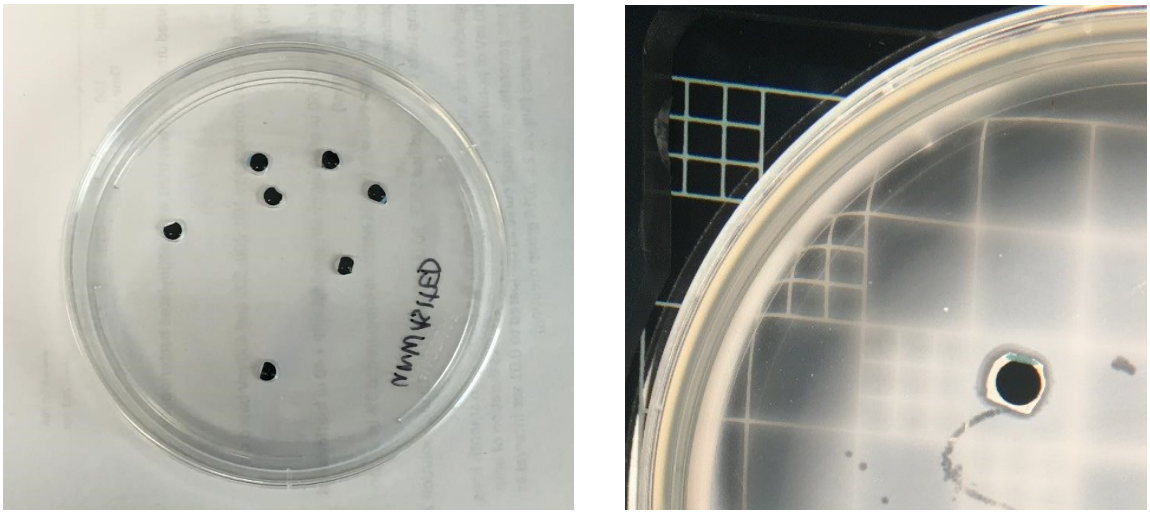

Figure S3. Agar diffusion test of bacteriophage-modified SPCE. (A) Biotinylated O179 bacteriophages were immobilized onto the surface of the trimmed activated working electrode (WE) **(B)** After several washing and incubation steps prior to agar diffusion assay, WE showed zone of the clearing.

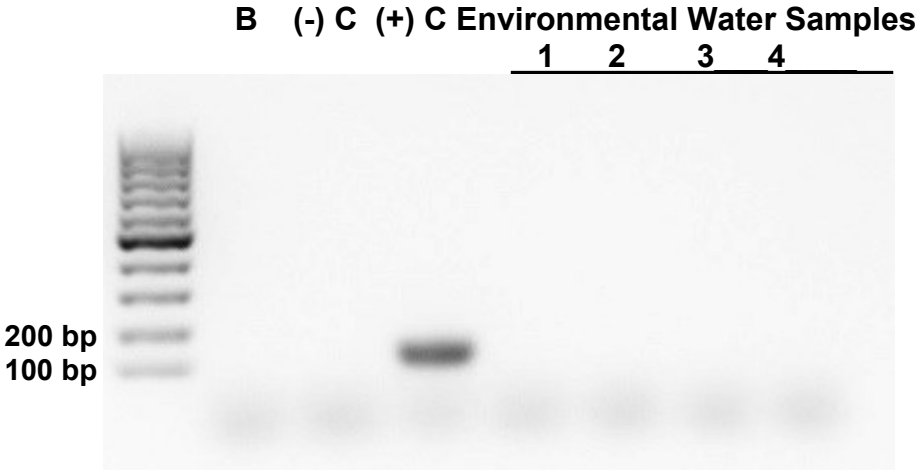

Figure S4. Gel image showing four environmental samples after conventional PCR targeting *stx1* gene. None of the samples showed bands for *stx1*(119-bp) gene**.** B-Blank, (-) C-Negative Control (*S.* Typhimurium), (+)C-Positive Control (O157:H7), 1-Site 1(P1-D), 2-Site 2(P8-W), 3-Site 3(P1-W) and 4-Site 4(P7-W)

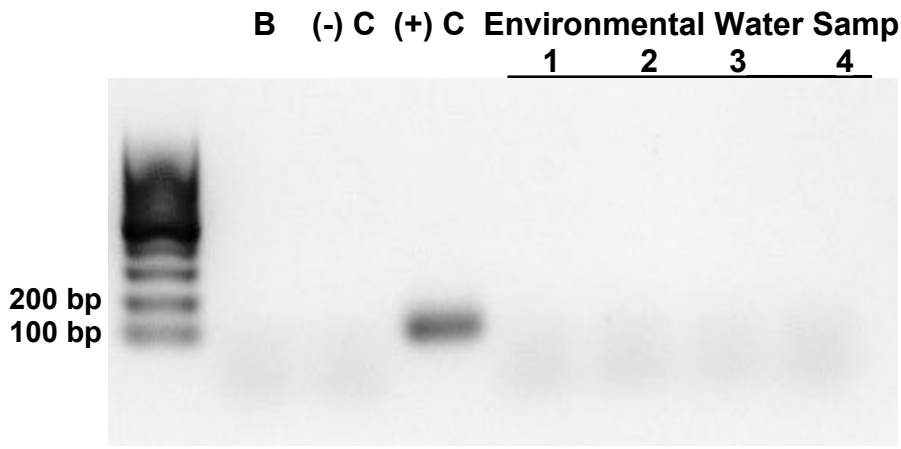

Figure S5. Gel image showing four environmental samples after conventional PCR targeting *stx2* gene. None of the samples showed bands for *stx2* (104-bp) gene**.** B-Blank, (-) C-Negative Control (*S.* Typhimurium), (+) C-Positive Control (O157:H7), 1-Site 1(P1-D), 2-Site 2(P8-W), 3-Site 3(P1-W) and 4-Site 4(P7-W)

References

1. I. A. Quintela, B. G. de los Reyes, C.-S. Lin and V. C. Wu, *Nanoscale*, 2015, **7**, 2417-2426.