

An EIS Biosensing Platform Using Co-immobilized, Indigenous, Target-Specific Bacteriophages for Dual-Pathogen Detection

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Foodborne pathogens such as *Escherichia coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa* threaten food safety, yet conventional detection methods such as culture, ELISA, and PCR remain slow and costly. This contribution presents a label-free electrochemical impedance spectroscopy (EIS) biosensing platform that uses co-immobilized, target-specific bacteriophages—indigenous Philippine isolates ev_vro1 (USTCMS 088.25), sv_ver1 (USTCMS 077.25), and pv-cmp3 (USTCMS 066.25), specific to *E. coli*, *S. Typhimurium*, and *P. aeruginosa*, respectively—for rapid dual-pathogen screening on a Zensor™ gold screen-printed electrode (Au-SPE). The platform builds on foundational single-pathogen sensors, in which individual host-specific phages on Au-SPEs detected their targets down to 10¹ CFU/mL for *S. Typhimurium* and *P. aeruginosa*, thereby establishing the chemistry that was later extended to dual-pathogen cocktails. The platform was demonstrated using two optimized 50:50 phage cocktails that share *E. coli* as a common target: an *E. coli*/*S. Typhimurium* cocktail and an *E. coli*/*P. aeruginosa* cocktail. Phages were covalently coupled to a mixed self-assembled monolayer via EDC/NHS crosslinking; immobilization was confirmed by FTIR and Nyquist-plot analysis using a [Fe(CN)₆]^{3-/4-} redox probe, with charge-transfer resistance (R_{ct}) as the analytical signal. Under optimized conditions, detection was achieved within 5 minutes. Across the four target-cocktail combinations, the sensors showed strong linearity ($R^2 \geq 0.97$) over 10² or 10³ to 10⁸ CFU/mL, depending on the cocktail, with sensitivities of 2564 and 2271 $\Omega/\log(\text{CFU/mL})$ for *S. Typhimurium* and *E. coli*, respectively, in the first cocktail, and 685.55 and 441 $\Omega/\log(\text{CFU/mL})$ for *E. coli* and *P. aeruginosa* in the second, and limits of detection at the lowest validated concentration of each cocktail. The sensors were selective for their hosts over non-target bacteria, e.g., an approximately 127-fold higher response to *S. Typhimurium* than to *P. aeruginosa*, and were repeatable, with relative standard deviations of 6.4-7.3%. In mixed-target samples, the additive and overlapping R_{ct} responses currently preclude quantitative discrimination of individual analytes, so the platform functions as a rapid presence/absence early-warning screen. Quantitative multiplexing will be pursued through a spatially resolved array bearing one phage per working electrode, complemented by non-lytic phage constructs and improved surface stability. Overall, the co-immobilized indigenous phage-EIS platform offers a rapid, low-cost, and selective approach to multiplexed screening of foodborne pathogens for point-of-care food safety applications.

Keywords: Bacteriophage biosensor; Electrochemical Impedance Spectroscopy; Dual-pathogen detection; Screen-printed electrode; Food safety

References

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