Supplementary Information

*for*

# On-Site Amplification-Free Electrochemical Detection of Plant Pathogen *Xylella fastidiosa* via Cathodic Potential-Induced DNA Adsorption

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**Operational steps of xylem sap extractor device**

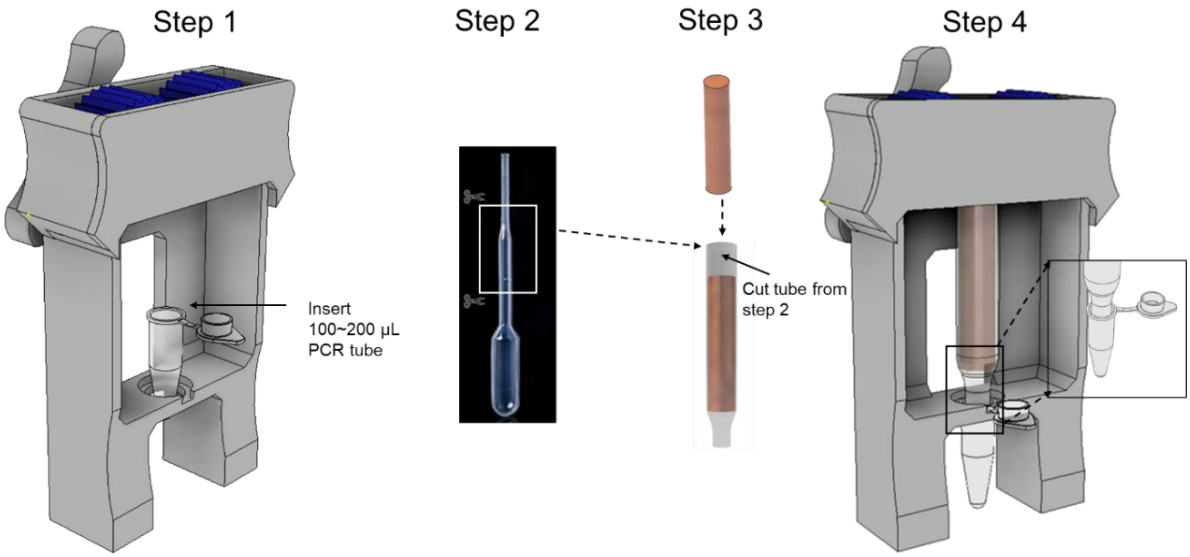


***Figure S1.*** *Operational steps 1–4 of the portable 3D-printed xylem sap extraction device. A 100–200 µL PCR tube is placed in the device to collect sap (Step 1). A 0.2–1 mL dropper, which prevents contamination, is prepared and cut as shown (Step 2). The vine stem is then fully inserted into the dropper (Step 3), and the dropper is loaded into the device and pressed onto the collection tube to extract the sap (Step 4).*

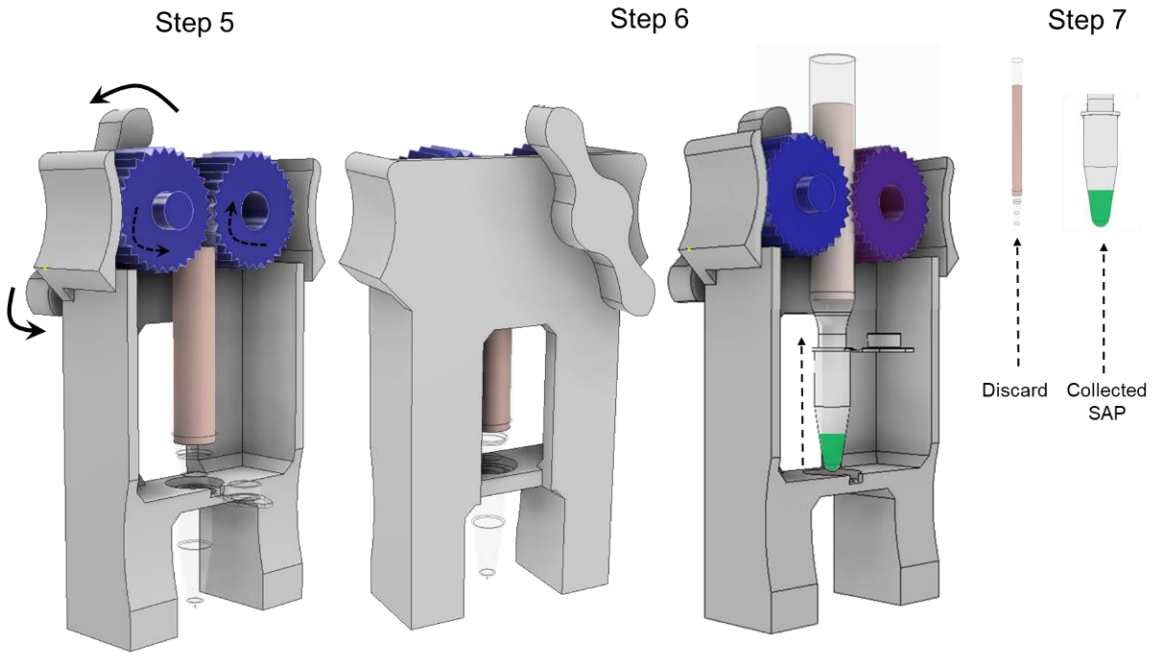


***Figure S2.*** *Operational steps 5–7 of the portable 3D-printed xylem sap extraction device. The mechanism, as shown in the figure, is activated by turning the handle counterclockwise to start squeezing the tube containing the stem (Step 5). As the vine stem is squeezed, it moves upward along with the tube, allowing sap to be collected in the PCR tube (Step 6). Once extraction is complete, the sap is collected in the tube and the dropper can be safely disposed of (Step 7).*

**Table S1.** Synthetic target, capture probe, primer sets, and non-specific target sequences used for *X. fastidiosa* electrochemical and PCR assays.

| Oligos | Sequences 5'-3' | Length (bp) |
|---|---|---|
| *X. fastidiosa* synthetic target | GTTTCGGGTTGCGTGGTGAAATCAAGATAG<br>AGTCTTGGACTGAGCCACGGGATGCGATCT<br>TCCGTTACCAGCCGTGGTTATTACGTTCTCC<br>TACGGGTACCGAGTCCATGTTGAATGGTGCCCGTG | 126 |
| *X. fastidiosa* Capture probe | GAACGTAATAACCACGGCTGGTAACGGAAGATC/Bio/ | 33 |
| Forward primer | CACGGCTGGTAACGGAAGA | 19 |
| Backward primer | GGGTTGCGTGGTGAAATCAAG | 21 |
| Non-specific target (NsT)<br>(*Xanthomonas albilineans*) | GATCTCGCGTATTGCCAGGGATATGGCGATCG<br>ATCTGCCCCTGGCCATGCTGTTCGAGCTGCCC<br>ACGGTAGCGCAGCTTAGCGAATCCCTCGCCAG<br>CCATGCACGCGACAGCGATTACGATGTCAT | 126 |

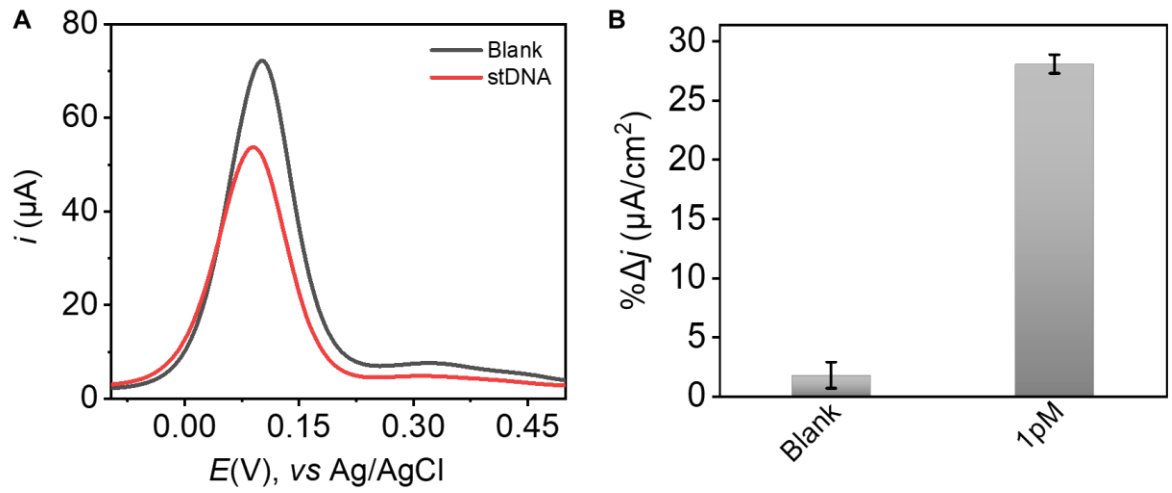


***Figure S3.*** *(A) Representative DPV responses in the presence and absence of 1 pM Xylella fastidiosa DNA, with the (B) corresponding bar graph showing the mean percentage decrease in current density relative to the blank (no-target). Error bars represent the SD from independent measurements performed using separately prepared electrodes.*

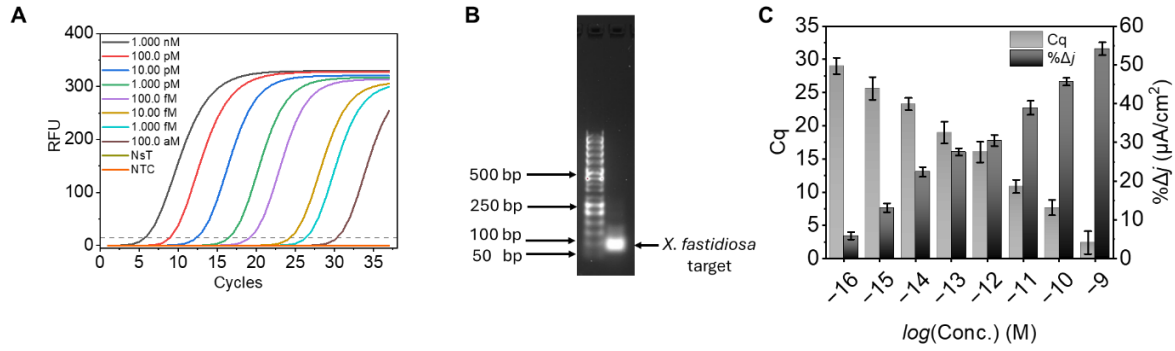


***Figure S4.*** *(A) qPCR validation of the electrochemical assay using synthetic Xylella fastidiosa stDNA spiked into vine xylem sap. PCR amplification was performed on samples containing 100 aM–1.0 nM synthetic DNA and (B) gel electrophoresis of the amplified products confirmed the presence of the target DNA. (C) The correlation between the percentage decrease in average current density from DPV measurements and the corresponding Cq values obtained by standard PCR for samples containing 100 aM–1.0 nM synthetic stDNA. Error bars represent the SD from three independent DPV and PCR measurements.*

**Table S2:** Comparison of the current method with conventional *X. fastidiosa* DNA detection methods and established biosensing platforms.

| Methods | Detection Limit | Sample to answer time (min) |
|---|---|---|
| This work (EC sensing) | 100 aM (~300 copies/µL) | ~30 |
| Real-time PCR[S1] | ~10 copies | ~120 |
| LAMP[S1] | ~250 copies | ~60 h |
| Conventional PCR[S1] | ~500 copies | ~180 |
| Electrolyte-gated transistor[S2] | 2 ± 1 bacteria in 0.1 mL | ~30 |
| ELISA[S3] | ~$10^4$ CFU/mL | ~240 |
| Electrochemical Impedance Spectroscopy[S3] | $1.3\times10^3$CFU/ml | ~60 |

S1. S. J. Harper, L. I. Ward and G. R. G. Clover, *Phytopathology*, **2010**, *100*, 1282–1288.
S2. L. Sarcina, E. Macchia, G. Loconsole, G. D'Attoma, P. Bollella and M. Catacchio *et al.*, *Adv. Sci.*, **2022**, *9*, 2203900.
S3. M. S. Chiriacò, A. Luvisi, E. Primiceri, E. Sabella, L. De Bellis and G. Maruccio, *Sci. Rep.*, **2018**, *8*, 7376.