## SUPPORTING INFORMATION

# Nanotechnology in Plant Disease Management: DNA-Directed Silver Nanoparticles on Graphene Oxide as an Antibacterial Against *Xanthomonas Perforans*

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#### Synthesis of DNA

Oligonucleotides, including DNA-1: 5' AAT GTG CTC CCC CA<u>GCGCGCTT</u>-FITC-3' and DNA-2: 3' TTA CAC GAG GGG GT -5', were synthesized on an ABI 3400 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA, USA) at the 1 µmol scale. The nucleotides and FITC-labeled CPG were purchased from Glen Research (Sterling, VA, USA). Deprotection was performed in AMA (ammonium hydroxide/ 40% aqueous methylamine 1:1) at 65 °C for 30 min, and deprotected sequences were purified by reversed-phase HPLC (ProStar, Varian, Walnut

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Creek, CA, USA) with a C18 column (Econosil, 5  $\mu$ m, 250-4.6 mm) from Alltech (Deerfield, IL, USA). The mobile phase was a mixture of 100 mM triethylamine-acetic acid buffer (TEAA, pH 7.5) and acetonitrile (0-30 min, 10-100%). Immediately after HPLC purification, the purified DNA solution was dried in acid-resistant centriVap centrifugal vacuum concentrators (Labconco, Kansas City, MO, USA). The dried DNA was dissolved in 50  $\mu$ L DNA grade water, and the concentration of each sequence was determined based on the absorbance value at 260 nm using an 1800 UV-Vis (Shimadzu Scientific Instruments, Columbia, MD, USA).

#### Preparation of Bacterial Culture for in vitro Experiments

*X. perforans* race T4 strain Xp-F7 isolated from tomato in Quincy, FLwas used in the study. Bacterial culture stored in 30% glycerol at -80°C was plated on Nutrient Agar (NA) media plates and incubated at 28°C for 48 h to check for purity. Pure colonies were further plated on NA and incubated as described above. Bacterial colonies from NA were suspended in 10 mL sterile dH<sub>2</sub>O and adjusted to OD<sub>600 nm</sub>= 0.6 (SmartSpect Plus UV spectrometer, Bio-Rad, Philadelphia PA, USA). A 100 µL volume of the bacterial suspension was added to 0.7 mL of sterile Nutrient Broth (NB) in a 10 mL sterile plastic tube. The bacterial suspension in NB was exposed to 0.2 mL of NPs to achieve the final concentrations described in the experimental section. The bacterial culture was incubated at 28°C and 250 rpm (Forma Refrigerated Stackable Orbital Shaker, Thermo Electron Corporation, Asheville, NC, USA) for 20 h for TEM and FESEM experiments and 1,5 10, 20 and 60 min for time-dependent fluorescence microscopy experiments. For bacterial viability experiments, the bacterial suspension at 20 h was transferred to a sterile 1 mL microfuge tube and centrifuged at 15,000 rpm for 3 minutes. The supernatant was discarded, and the cells were resuspended in sterile dH<sub>2</sub>O and washed twice. The bacterial enumeration was determined by the standard plating method. A 100  $\mu$ L volume of the sample was taken to initiate a 10-fold dilution series (10<sup>-1</sup> to 10<sup>-7</sup>), and 100  $\mu$ L of each dilution was spread on yeast nutrient agar (YNA) and incubated at 28°C for 48 h, followed by colony counts. Each treatment in every experiment had two or three replications, and all the experiments were performed twice.

#### **SEM Analysis**

The morphologies of healthy cells, as control, without Ag@dsDNA@GO composite treatment and with Ag@dsDNA@GO composite treatment was studied with SEM. (Figure S1). Normal *X. perforans* cells deposited on poly-L-lysine- treated 0.2 micron Millipore filters and a glass surface are shown (Supplementary Figure S1a and S1b). After 20 h exposure to 20 ppm Ag@dsDNA@GO, the membrane of *X. perforans* cells was completely destroyed with severe deformation, as indicated (Supplementary Figure S1c and S1d).

### **Supplementary figures**



**Figure S1.** Scanning electron microscopy analysis of intact bacteria cells before and after treatment with Ag@dsDNA@GO composites. (a) SEM images of *X. perforans* cells before treatment (control) on poly-L-lysine-treated 0.2 micron Millipore filters. (b) Before treatment (control) on a glass surface. (c) After treatment with 20 ppm Ag(18nm)@dsDNA@GO composite for 20 hours on poly-L-lysine-treated 0.2 micron Millipore filters. (d) After treatment with 20 ppm Ag(18nm)@dsDNA@GO composite for 20 hours on poly-L-lysine-treated 0.2 micron Millipore filters. (d) After treatment with 20 ppm Ag(18nm)@dsDNA@GO composite for 20 hours on a glass surface.



**Figure S2.** Growth inhibition of *X. perforans* strain Xp-F7 upon exposure to bare AgNPs and Ag@dsDNA@GO composites. (a) Antibacterial activity of ~Ag)8nm)@GO composite without DNA towards *X. perforans* strain Xp-F7 with respective concentrations of 1, 5, 20,100, 50, 200 ppm. Column means labeled with different letters are statistically significantly different at  $P \le 0.005$  based on the Student Newman Keuls Test. The error bars represent the standard error of the mean.



**Figure S3.** Growth inhibition of *X. perforans* strain Xp-F7 upon exposure to bare AgNPs and Ag@dsDNA@GO composites. (a) Antibacterial activity of ~5 nm Ag NPs grown on dsDNA with respective concentrations of 10, 20, 30, 40, 50, 100 and 200 ppm. (b) Antibacterial activity of Ag(5 nm)@dsDNA@GO nanostructures with respective concentrations of 0 (control), 1, 4, 8, 12, 16 and 20 ppm. Column means labeled with different letters are statistically significantly different at  $P \le 0.005$  based on the Student Newman Keuls Test. The error bars represent the standard error of the mean.