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# Supplementary Information

## Synthesis of Organic-Inorganic Hybrid Nanocomposites Modified by Catalase-like Catalytic Sites for the Controlling of Kiwifruit Bacterial Canker

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## Supplementary Experimental Section

### Characterizations

The morphology of the samples was observed by scanning electron microscopy (SEM; Nova NanoSEM$^{TM}$ 450, FEI, USA) and transmission electron microscopy (TEM; Talos F200C, FEI, USA), and elemental analysis was performed by using an energy dispersive spectroscopy (EDS, Oxford Instruments, X-MAXn65 T, U.K.). We also characterized the structure and composition of the samples by X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Scientific, USA). All electron spin resonance (ESR) measurements were carried out using a Bruker EMX ESR spectrometer (Billerica, MA) at ambient temperature. Fourier Transform infrared spectroscopy (FT-IR) were recorded in the range of 4000 – 400 $cm^{-1}$ with 32 scans at a resolution of 2 $cm^{-1}$ using FT-IR spectrometer ((Nicolet IS50, ThermoFisher, USA) at room temperature. The zeta potential and size distribution of the samples were determined through a laser diffraction particle size analyzer (DLS, Nano Brook Omni, USA).

### Peroxidase (POD)-like activity of nanocomposites.

Steady-state kinetics assays were conducted at 25 °C in 200 μL of phosphate buffer saline solution (PBS, pH 4.5) with 20 μL of ZnO@PDA-Mn nanocomposites solution (final concentration 10 μg/mL) as catalyst in the presence of $H_2O_2$ and 3, 3', 5, 5'-tetramethylbenzidine (TMB). 1) $H_2O_2$ as the substrate: 5 μL of 0.0416 M TMB and different amounts of $H_2O_2$ (5 μL of 0.12%,5 μL of 0.24%, 5 μL of 0.47%,5 μL of 0.94%,5 μL of 1.88%,5 μL of 3.75%,5 μL of 7.5%, 5 μL of 15% $H_2O_2$) were added to the reaction system containing ZnO@PDA-Mn nanocomposites, with change in absorbance recorded at 652 nm with time at 25°C. 2) TMB as the substrate: 5μL of 15% $H_2O_2$ and different amounts of TMB (5 μL of 0.0052 M, 5 μL of 0.0104 M, 5μL of 0.0208 M, 5, 10, 15, 20, 25 μL of 0.0416M of TMB solution) were added to the reaction system containing ZnO@PDA-Mn nanocomposite, with the change in

absorbance recorded at 652 nm with time at 25 °C. For comparison, the enzyme-like activities of PDA, ZnO NPs and ZnO@PDA nanocomposites were also measured.

The apparent kinetic parameters were calculated based on Michaelis equation (10.1016/j.biomaterials.2010.11.004).

$$v = V_{max} \times [S]/(K_m + [S])$$

$v$ is the initial velocity, $V_{max}$ is the maximal reaction velocity, [S] is the concentration of substrate and $K_m$ is the Michaelis constant.

**Superoxide dismutase (SOD)-like activity of nanocomposites.**

The SOD-like activity of the ZnO@PDA-Mn nanocomposites was evaluated at room temperature using a commercial colorimetric SOD assay kit from Solarbio, in accordance with the manufacturer's instructions. The SOD-like activity of ZnO@PDA-Mn (10 μg/mL and 15 μg/mL) was expressed as the percentage inhibition of the competitive WST-1 reaction with superoxide by natural SOD enzymes.

**Determination of Hydroxy radical (•OH).**

·OH measurement. Glass capillary tubes containing ZnO@PDA-Mn (0.1 mg/mL, 50 μL), $H_2O_2$ (0.001 M, 50 μL), 5-tert-butoxycarbonyl 5-methyl-1-pyrroline-N-oxide (BMPO, 10mM), and PBS (pH 4.5) were inserted into the electron spin resonance (ESR) cavity to record ·OH signals at selected times.

**The interaction between nanoparticles and bacterial surfaces was observed by TEM.**

Approximately 5 mL of the bacterial suspension ($1\times10^9$ CFU/mL) was centrifuged at 6000 rpm at 4 ℃ for 1.5 min. After discarding the supernatant, the bacteria were washed with 1 mL PBS thrice to remove the medium; finally, 1 mL PBS was added to resuspend the bacterial solution ($5\times10^9$ CFU/mL). In addition, 100 μL of the bacterial solution was mixed with 5 mL of the samples solution and incubated at 28 ℃ for 4 h. Thereafter, 20 μL aliquots were dropped on a carbon film coated with a copper grid (200 mesh), dried with filter paper for 1 min, and stained with uranium acetate (1%)

for 10 s. The excess uranium acetate was absorbed using a filter paper, and the samples were dried at room temperature, following which they were observed and appropriate images recorded using TEM.

**Live/dead cell staining**

Bacterial suspensions treated by $H_2O_2$, ZnO, ZnO@PDA-Mn and ZnO@PDA-Mn $+H_2O_2$ for 4 h. Then the bacterial suspensions were collected by centrifugation at 4000 r/min for 5 min. The cells were then stained with SYTO 9 and propidium iodide (PI) for 30 min and visualized via fluorescence microscopy (FluoroMax®–4P, HORIBA, France).

**Zone of inhibition (ZOI) assay**

ZnO@PDA-Mn were studied for the antibacterial efficacy against *Psa* using disk diffusion technique commonly known as Kirby Baurer method. This is a qualitative technique to test the ability of a material to inhibit microbial growth by measuring ZOI. Overnight bacterial culture was grown at 37 °C and its cell density was adjusted to $10^8$ CFU/mL (i.e. Optical density of 0.1 at 600 nm). 0.1 mL of bacterial culture was spread onto sterile agar plates. Plates were allowed to dried followed by placing 6 mm sterile disc soaked for 10 min in Zn-Th, ZnO and ZnO@PDA-Mn concentration 3.13 μg/mL. Post incubation at 37 °C for 48 h, ZOI around the disc was measured.

**Kiwifruit tissue section observation experiment**

After measuring the length of kiwifruit lesions, the kiwifruit tissues treated with different samples were sliced. The tissue sections were then immersed in 2.5% glutaraldehyde (Sigma-Aldrich, USA) and fixed for 10 hours at 4 °C under dark conditions. Subsequently, the tissue sections underwent dehydration with a series of ethanol concentrations (30%, 50%, 70%, 90%, and 100%) for 10 minutes each. Finally, the dried tissue sections were sputter-coated with gold for imaging using scanning electron microscopy.

**Table S1.** Comparison of Michaelis-Menton constants ($K_m$) and maximum reaction rates ($V_{max}$) of reported catalase-like nanozymes.

| Catalysts | $K_m$ (mM) | $V_{max}$(mg/(L·min)) | Ref. |
|---|---|---|---|
| ZnO@PDA-Mn | 116.3 | 6.03 | This work |
| Catalase | 249.4 | 5.76 | 10.1016/j.cej.2020.124249 |
| $Fe^{3+}$/nucleotide CPs | 112.2 | 4.61 | 10.1016/j.cej.2020.124249 |
| $RuO_2$ NP | 400.0 | / | 10.1002/chem.201200643 |
| Pt-Ft | 420.6 | $1.6\times 10^3$ | 10.1016/j.biomaterials.2010.11.004 |
| Au-Si-ACD | 117.0 | 0.23 | 10.1002/smll.201603051 |
| N-PCNSs-3 | 66.3 | 1.03 | 10.1038/s41467-017-02317-2 |
| IrNCs | 132.0 | / | 10.1016/j.biomaterials.2018.07.049 |
| CN | 393.3 | 4.76 | 10.1021/acs.nanolett.9b01333 |
| $Au_{24}Ag_1$ | 222.4 | 2.21 | 10.1021/acs.nanolett.0c05148 |
| *pero*-nanozysome | 90.0 | 4.67 | 10.1002/adfm.202007130 |
| $IrO_x$ NPs | 188.0 | 10.82 | 10.1002/anie.201916142 |

**Table S2.** Abbreviations

| Abbreviation | Full name |
|---|---|
| *Xac* | *Xanthomonas axonopodis* pv.*citr* |
| *Psa* | *Pseudomonas syringae* pv. *actinidiae* |
| OD | Optical density |
| CFU | Colony Forming Unit |
| POD | Peroxidase |
| CAT | Catalase |
| SOD | Superoxide dismutase |
| PBS | phosphate buffer saline solution |
| h | Hour |
| TMB | 3,3',5,5'-Tetramethylbenzidine |
| min | Minute |
| TEM | Transmission electron microscopy |
| SEM | Scanning electron microscopy |
| FT-IR | Fourier Transform infrared spectroscopy |
| XPS | X-Ray Photoelectron Spectroscopy |
| XRD | X-Ray Diffraction |
| DLS | Dynamic light scattering |

| | |
|---|---|
| EDS | Energy dispersive spectroscopy |
| ROS | Reactive Oxygen Species |
| PI | Propidium iodide |
| Zn-Th | Zinc Thiozole |
| PDA | Polydopamine |

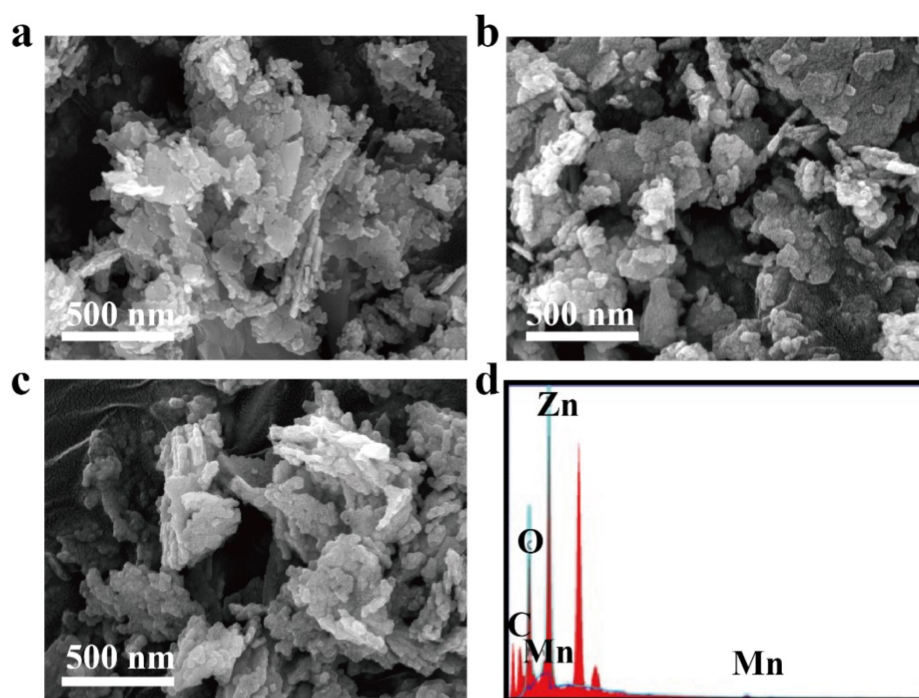


**Figure S1.** SEM and EDS characterization of (a)ZnO, (b)ZnO@PDA and (c,d)ZnO@PDA-Mn.

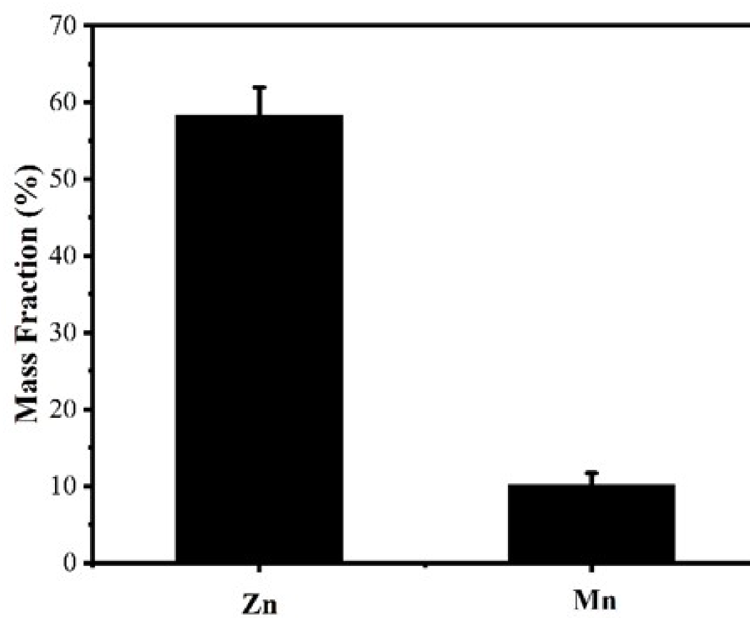


**Figure S2**. The Mass Fraction of Mn and Zn in ZnO@PDA-Mn.

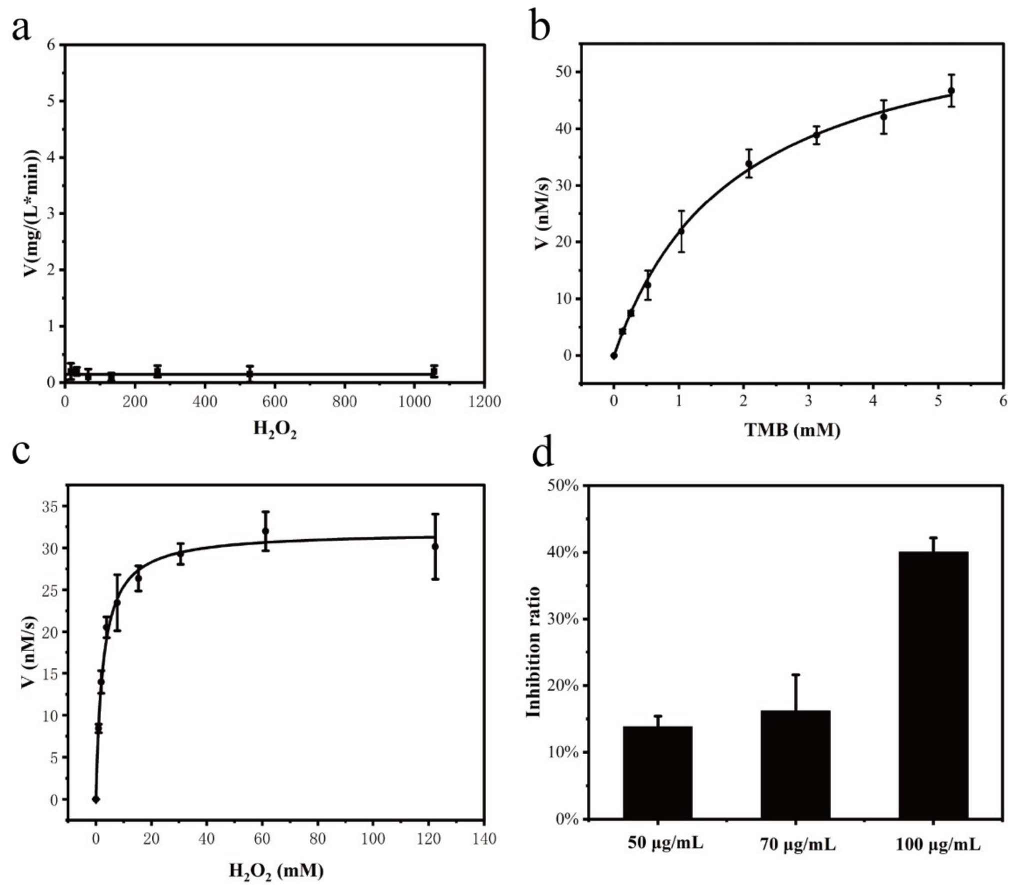

**Figure S3**. (a) Steady-state kinetics assay of CAT-like activity of PDA with varied $H_2O_2$. (b) Evaluation of the steady-state kinetics assay for the POD-like activity of PDA using varying concentrations of TMB. (c) Assessment of the steady-state kinetics assay for the POD-like activity of PDA using varying concentrations of $H_2O_2$. (d) Detection of SOD-like activity of PDA through WST-1 assay.

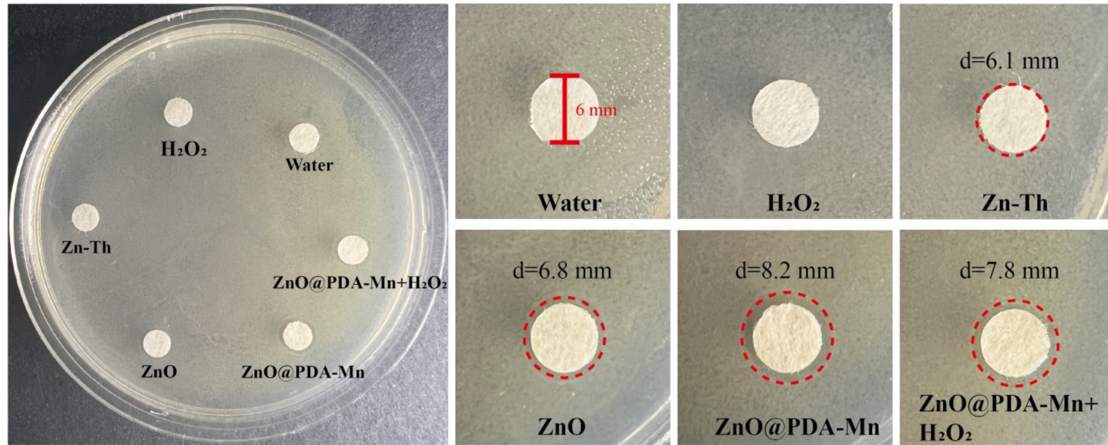


**Figure S4**. zone of inhibition assay.

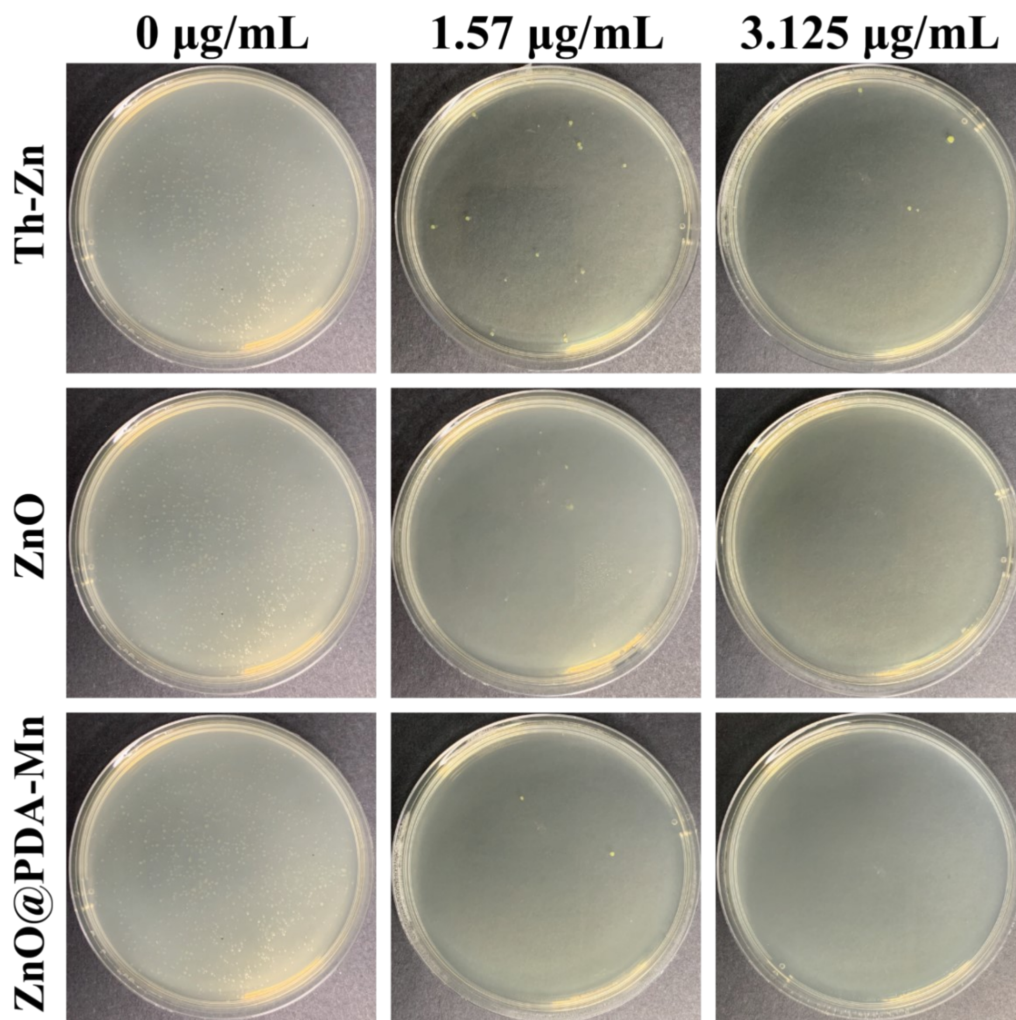


**Figure S5.** Representative colony formation of *Xanthomonas axonopodis* pv. *citr* (*Xac*) bacteria in different treatment groups.