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Supplementary Material

Metal-phenolic networks as tunable spore coat mimetics

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1. Experimental section:

1.1 Chemicals and Reagents. All chemicals were reagent grade and used without further purification unless otherwise stated. Tannic acid (99%), gallic acid monohydrate (98%), L-ascorbic acid (99%), iron (III) chloride ($FeCl_3$, 97%), aluminum chloride (99.9%), zinc sulfate heptahydrate (99%), manganese sulfate monohydrate (99%), 3-(N-morpholino) propanesulfonic acid (MOPS, 99.5%), sodium hydroxide (98%), hydrochloride acid (37%), acetone (99.5%), D-(+)-trehalose dihydrate (99%), sodium phosphate dibasic (99%), sodium phosphate monobasic (99%), and citric acid (99.5%) were purchased from Sigma-Aldrich. Absolute ethanol (molecular biology grade), sodium chloride, and agar (granulated) were purchased from Fisher BioReagents. Nutrient broth (microbiologically tested) powder was purchased from Fluka. Green tea extract (50% EGCG) was purchased from Bulk Supplements. Ultrapure water was generated from an ELGA PURELAB Quest UV (Model number: PQDIUVM1NSP). LIVE/DEAD™ BacLight™ Bacterial Viability Kit was purchased from ThermoFisher. Nutrient broth liquid media were prepared with 8 g of nutrient broth powder in 1L of nanopure water (adjust pH to 7.0 with sodium hydroxide) and used after autoclaving (20 min, 121° C). Nutrient agar plates were prepared on Petri dishes with 20 mL of nutrient agar solution (8 g of nutrient broth powder, and 15 g of agar in 1L of nanopure water, Adjusting pH to 7.0).

1.2 Analysis and Measurement. Optical density or absorbance was measured with a NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo Scientific, USA) or a Biotek synergy mx microplate reader (BioTek Instruments, USA). The cells were observed with a Revolve Fluorescence Microscope (Echo, USA).

1.3 Bacterial Strains and Culture. Strain (*Bacillus subtilis* (Ehrenberg) Cohn (ATCC 6051 LOT: 70044049) was used in this study. Cells were prepared for MPN coating as follows: *B. subtilis* strains (20% glycerol) stored at −80 °C were streaked onto nutrient agar plates and aerobically kept for ~18h at 30 °C. Single colonies were to inoculate nutrient broth. The cultures were kept for ~18 h at 30 °C and washed with nanopure water followed by centrifugation (6000 x g for 20 min, three times). After the final wash, cells were concentrated to a suspension (OD_{600} of 4.0, 4x stock) and were used immediately.

1.4 MPN Encapsulation. MPNs were coated on the surface of *B. subtilis* as previously published.[1] For different MPNs, equivalent amounts of tannic acid (1.6 mg mL^{-1}), gallic acid (1.5 mg mL^{-1}) or epigallocatechin gallate (2.7 mg mL^{-1}, 50% from tea extract) and an equal volume freshly-made aqueous solution of iron chloride (0.24 mg mL^{-1}), zinc sulfate heptahydrate (0.42 mg mL^{-1}), aluminum chloride (0.19 mg mL^{-1}), or manganese sulfate monohydrate (0.72 mg ml^{-1}) were applied for encapsulation.

1.5 Preparation of Lyophilized Cells. The lyophilization of uncoated and MPN-coated *B. subtilus* were prepared according to previous protocol.[1] Before use, the lyophilized cells were reconstituted in sterile 10 mM *L*-ascorbic acid solution or nutrient broth as necessary.

1.6 Bacterial Growth/Viability Assessment. Growth assays were performed in sterile 96-well plates, and OD_{600} was monitored by plate reader. Bacterial viability was determined by live/dead bacterial viability assay. Images were captured using fluorescence microscope for live (green fluorescence: λ_{ex}= 470/40 nm; λ_{em}= 525/50 nm) and dead cells (red fluorescence: λ_{ex}=560/40 nm, λ_{em}= 630/75 nm) with a 60X oil objective. Cell counts to determine the viable cells percentage were quantified by ImageJ from five randomly-chosen fields of view.

2. Supplemental Figures:

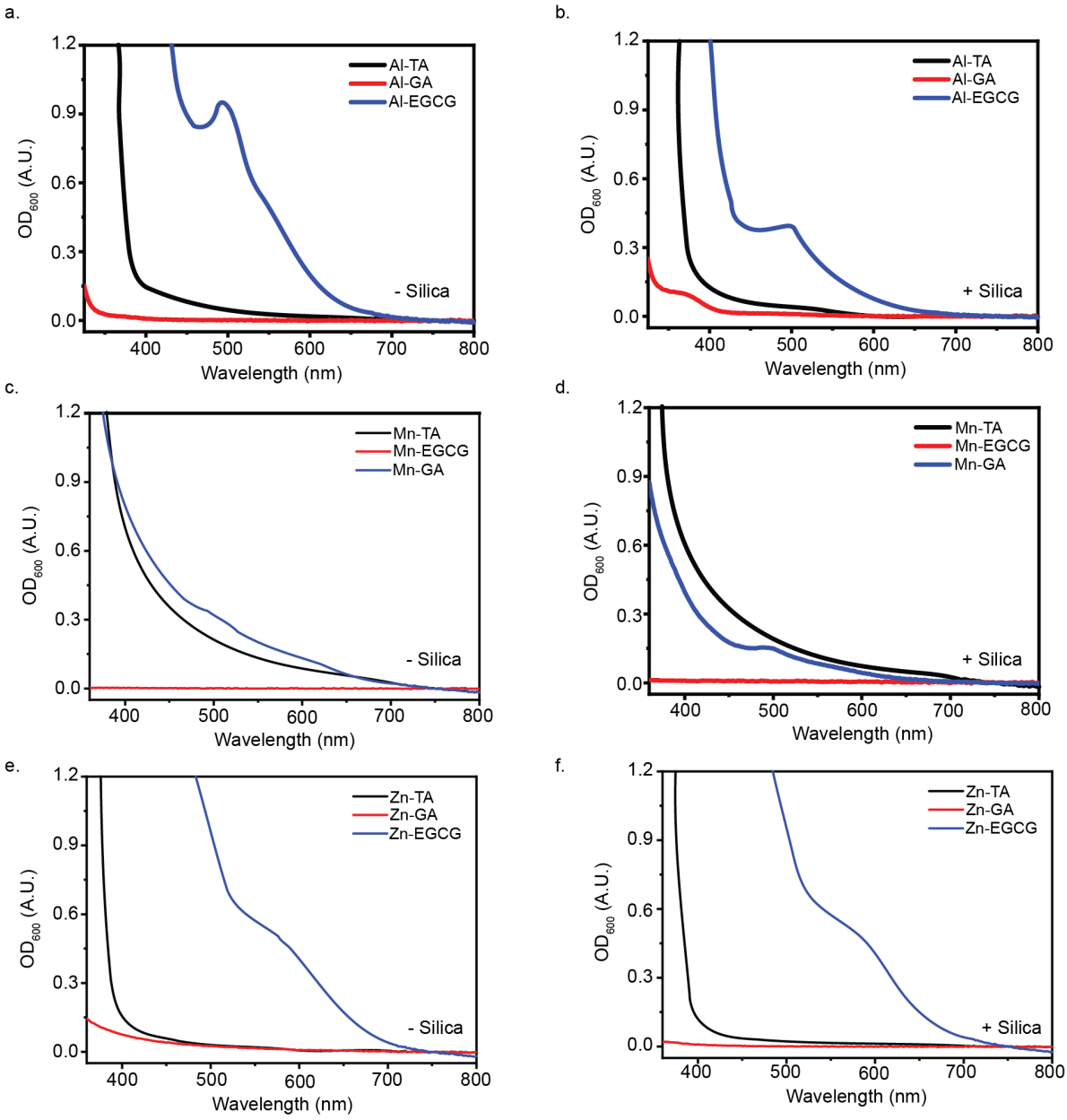

Figure 1. UV–vis spectra of metal-phenolic networks. UV-Vis absorbance of (a) Al^{III}-TA, Al^{III}-GA, and Al^{III}-EGCG complexes in MOPS buffer (10 mM, pH 7.5), (b) Al^{III}-TA, Al^{III}-GA, and Al^{III}-EGCG complexes after addition of silica particles (1mg/mL); (c) Mn^{II}-TA, Mn^{II}-GA, and Mn^{II}-EGCG complexes in MOPS buffer (10 mM, pH 7.5), (d) Mn^{II}-TA, Mn^{II}-GA, and Mn^{II}-EGCG complexes after addition of silica particles (1 mg/mL); (e) Zn^{II}-TA, Zn^{II}-GA, and Zn^{II}-EGCG complexes in MOPS buffer (10 mM, pH 7.5), (f) Zn^{II}-TA, Zn^{II}-GA, and Zn^{II}-EGCG complexes after addition of silica particles (1 mg/mL).

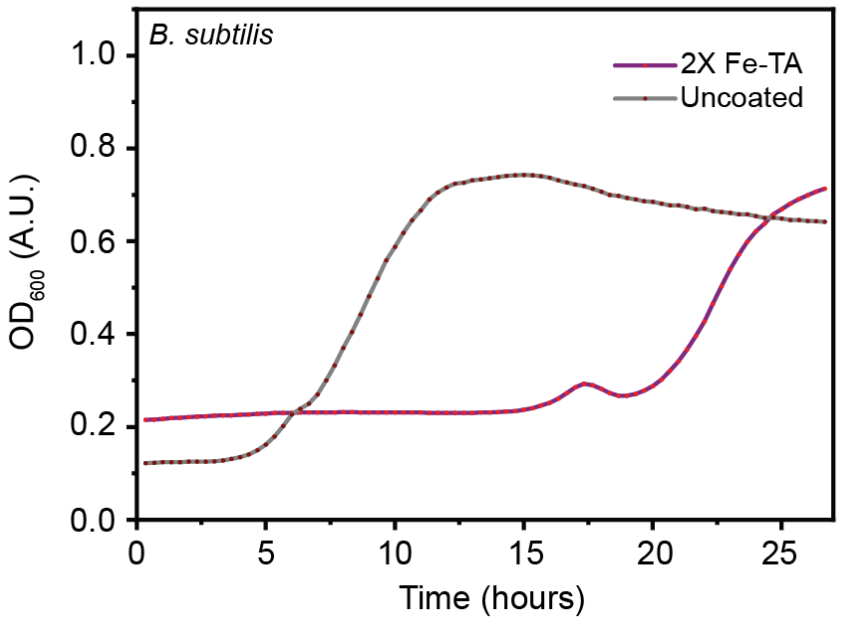

Figure 2. Growth curves monitored at OD_{600} of *B. subtilis* alone and MPN-coated *B. subtilis* ("2X" means coating is repeated twice).

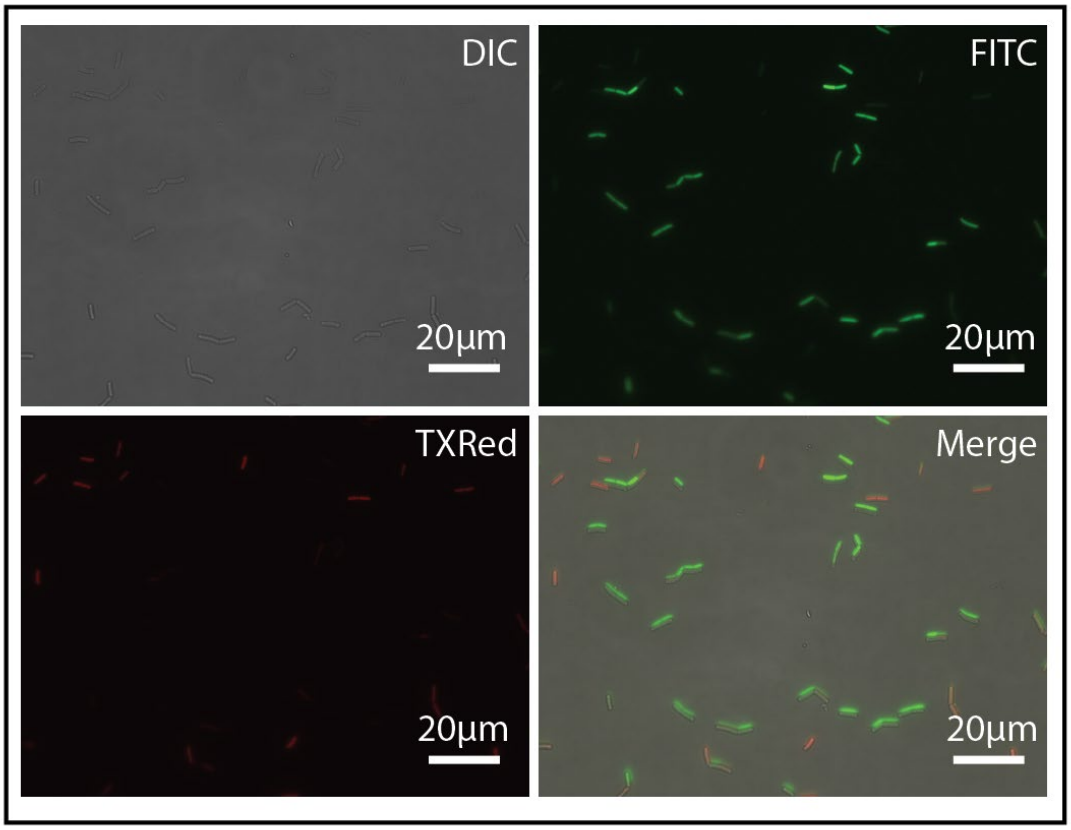

Figure 3. Bacterial viability assessment shows live/dead *B. subtilis* after Fe^{III}-TA encapsulation without addition of MOPS buffer (Viability: 54%). Representative composite images of DIC, green (live) and red (dead) fluorescence channels for *B. subtilis*.

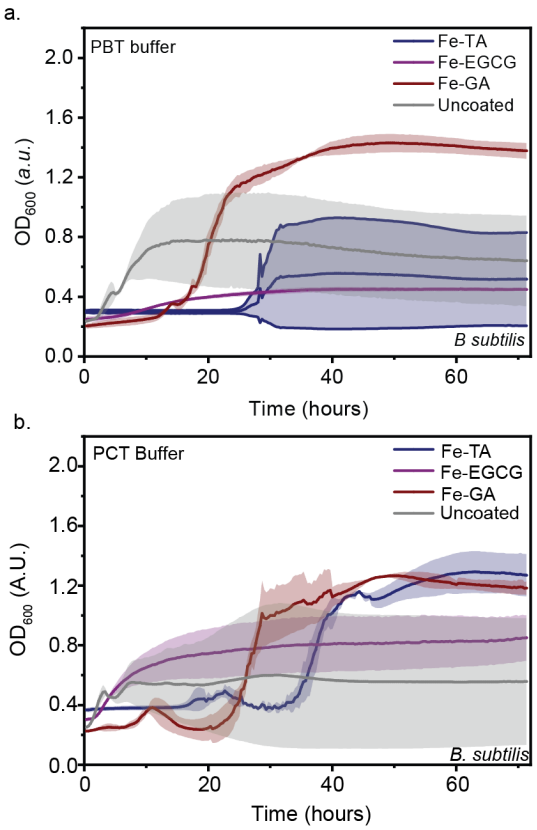

Figure 4. Growth curves monitored at OD_{600} of Fe^{III}-polyphenol-coated and uncoated *B. subtilis* following lyophilization with cryoprotectants (PB: phosphate-buffered saline; PC: phosphate-citrate buffer; cryoprotectant: 0.1 M trehalose). (Shaded region represents error bars represent SD for n=3 replicates)

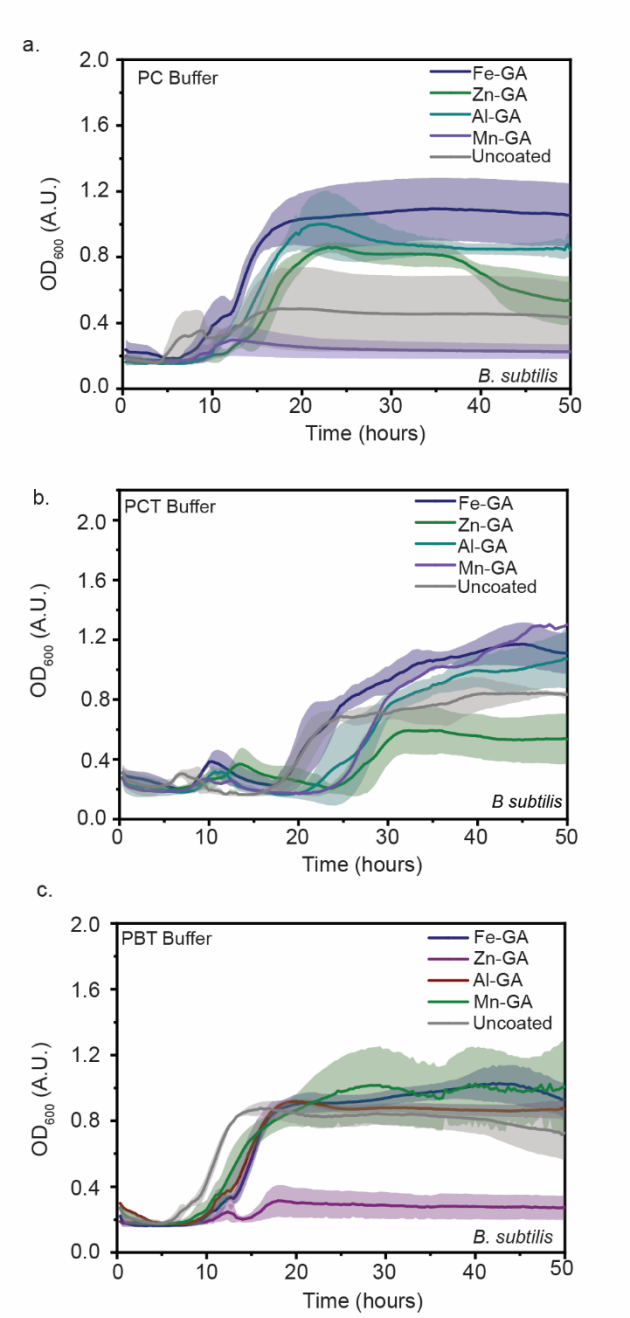

Figure 5. Growth curves monitored at OD_{600} of metal-GA-coated and uncoated *B. subtilis* following lyophilization with or without cryoprotectants (PB: phosphate-buffered saline; PC: phosphate-citrate buffer; cryoprotectant: 0.1 M trehalose). (Shaded region represents error bars represent SD for n=3 replicates)

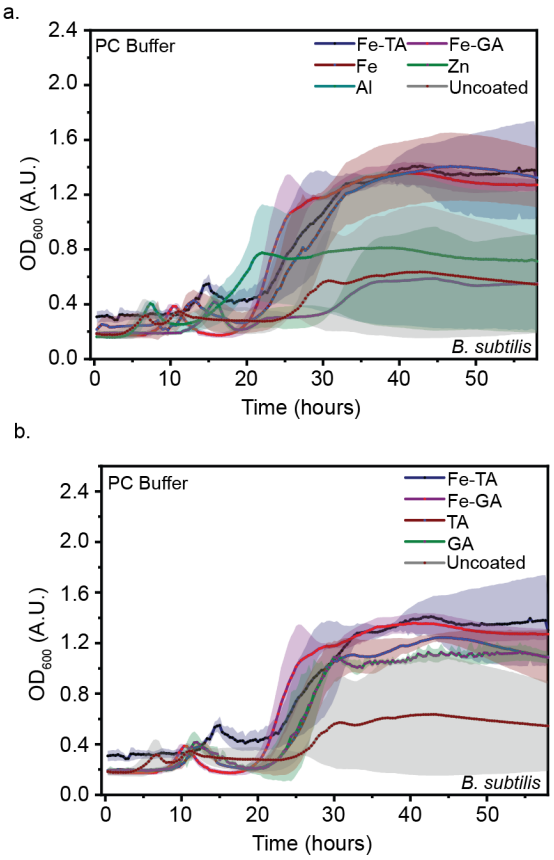

Figure 6. Growth curves monitored at OD_{600} of *B. subtilis* alone, with metals (Fe^{III}, Zn^{II}, and Al^{III}) only, with polyphenols (TA, GA) only, and with both (Fe-TA, Fe-GA-MPN-coated) following lyophilization in PC buffer. (Shaded region represents error bars represent SD for n=3 replicates)

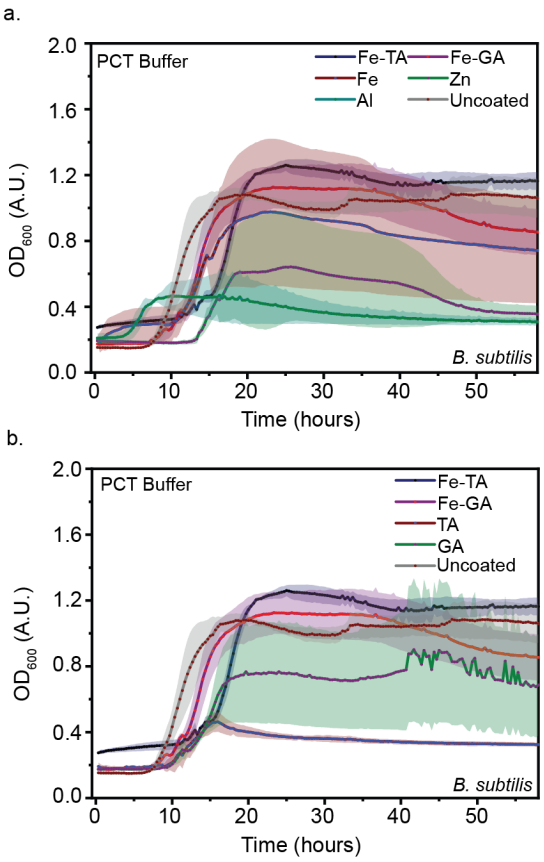

Figure 7. Growth curves monitored at OD_{600} of *B. subtilis* alone, with metals (Fe^{III}, Zn^{II}, and Al^{III}) only, with polyphenols (TA, GA) only, and with both (Fe-TA, Fe-GA-MPN-coated) following lyophilization in PC buffer supplement with 0.1 M trehalose. (Shaded region represents error bars represent SD for n=3 replicates)

3. References:

1 G. Fan, P. Wasuwanich, M. R. Rodriguez-Otero and A. L. Furst, *J. Am. Chem. Soc.*, 2022, **144**, 2438–2443.