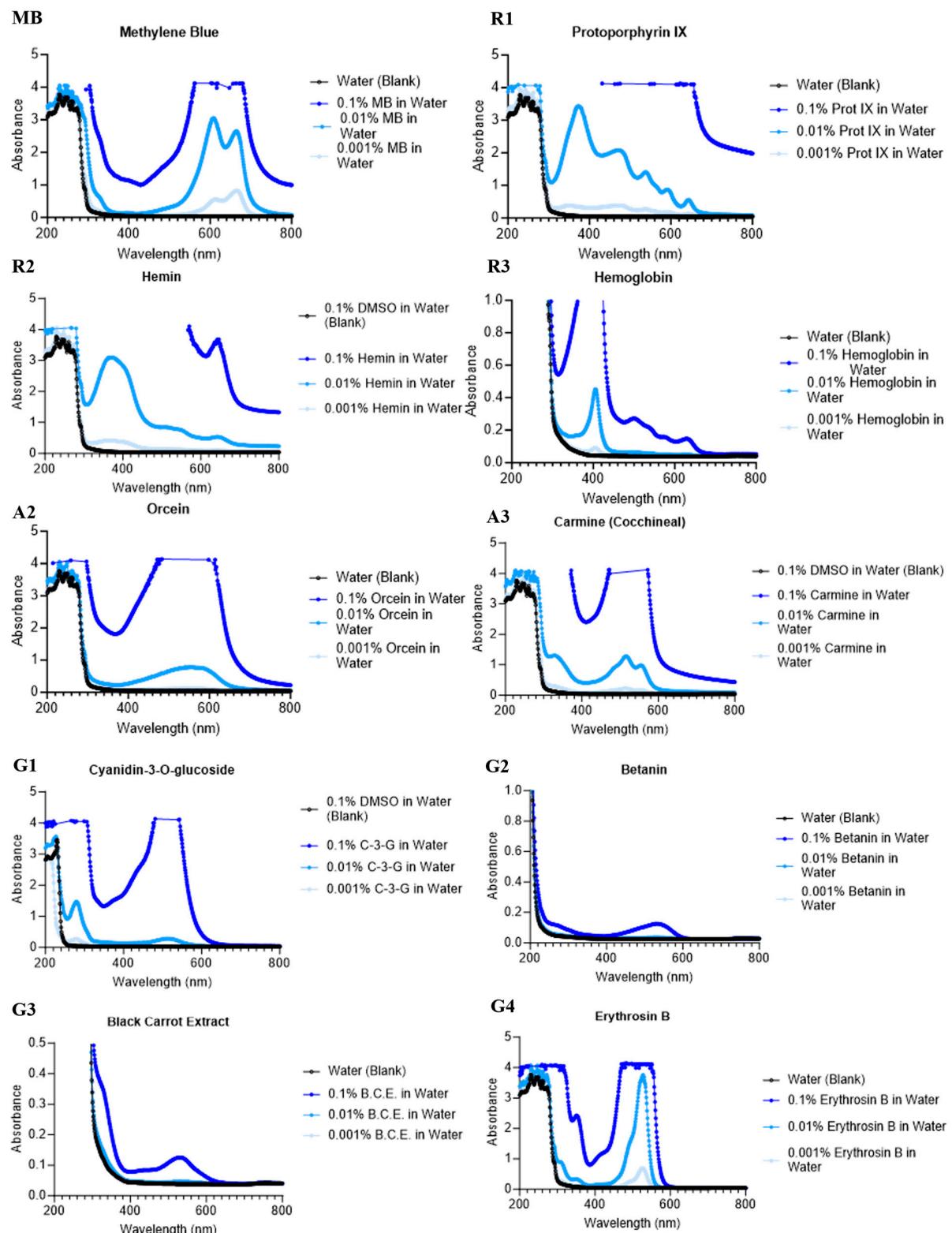
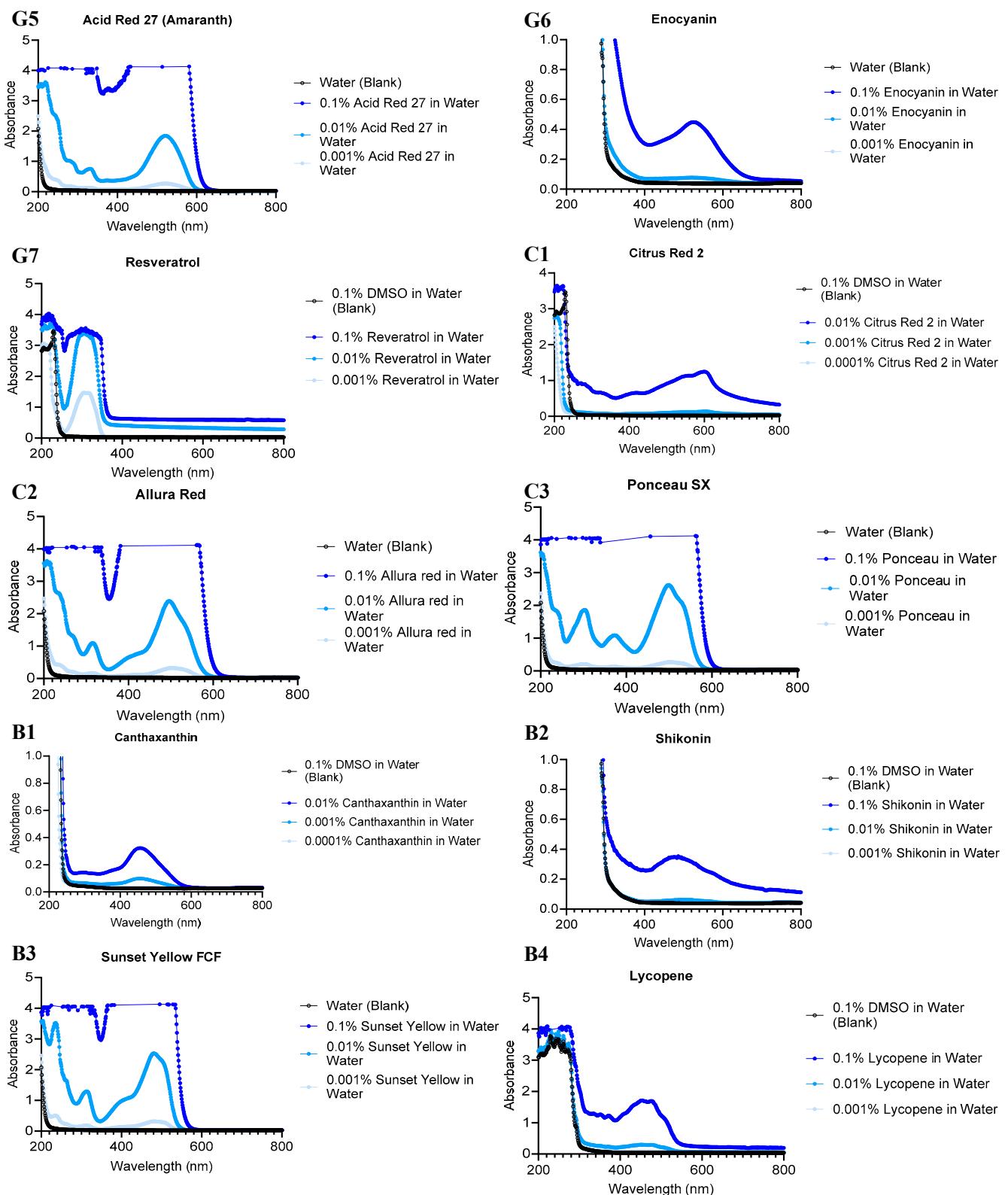


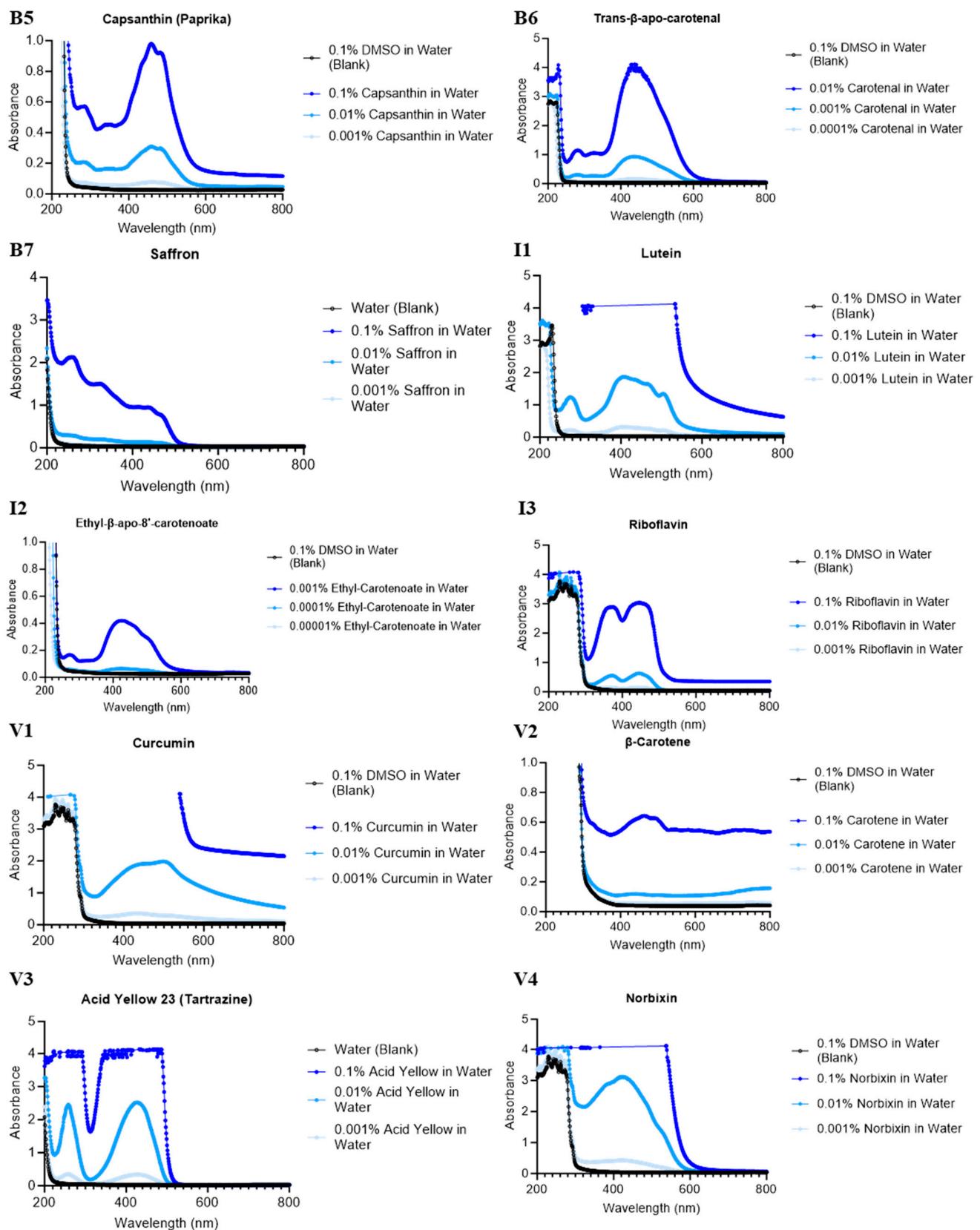
Photodynamic Inactivation of Foodborne Bacteria: Screening of 32 Potential Photosensitizers (*Supplementary Material*)



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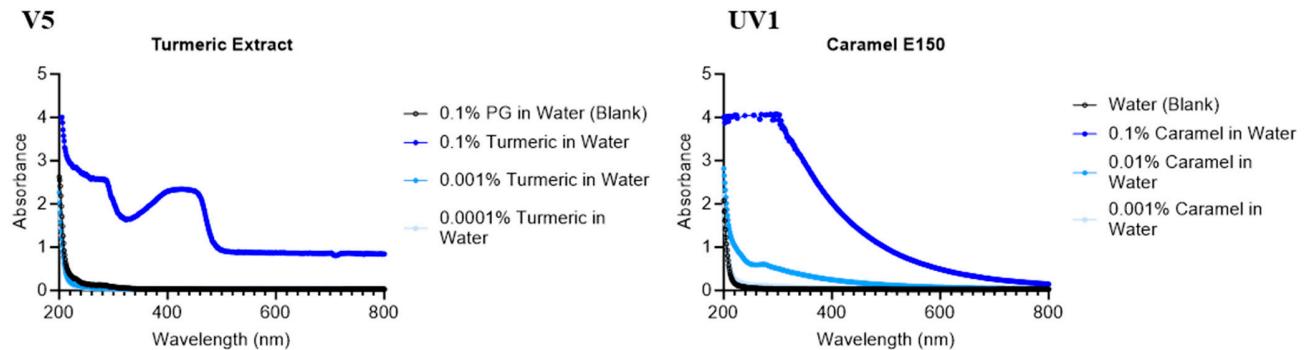


Figure S1. Spectral scans of photosensitizer candidates used in this study. The absorbance was measured over the wavelength range of 200 – 800 nm using a UV-Vis spectrophotometer. Here, **MB** corresponds to Methylene Blue, **R** corresponds to the photosensitizers used with red wavelength (630 nm), **A** corresponds to the photosensitizers used with amber wavelength (590 nm), **G** corresponds to the photosensitizers used with green wavelength (527 nm), **C** corresponds to the photosensitizers used with cyan wavelength (505 nm), **B** corresponds to the photosensitizers used with blue wavelength (470 nm), **I** corresponds to the photosensitizers used with indigo wavelength (445 nm), **V** corresponds to the photosensitizers used with violet wavelength (420 nm), and **UV** corresponds to the photosensitizers used with ultraviolet wavelength (365 nm). Titanium dioxide was not tested as it formed insoluble crystals and was used as a suspension in water instead.

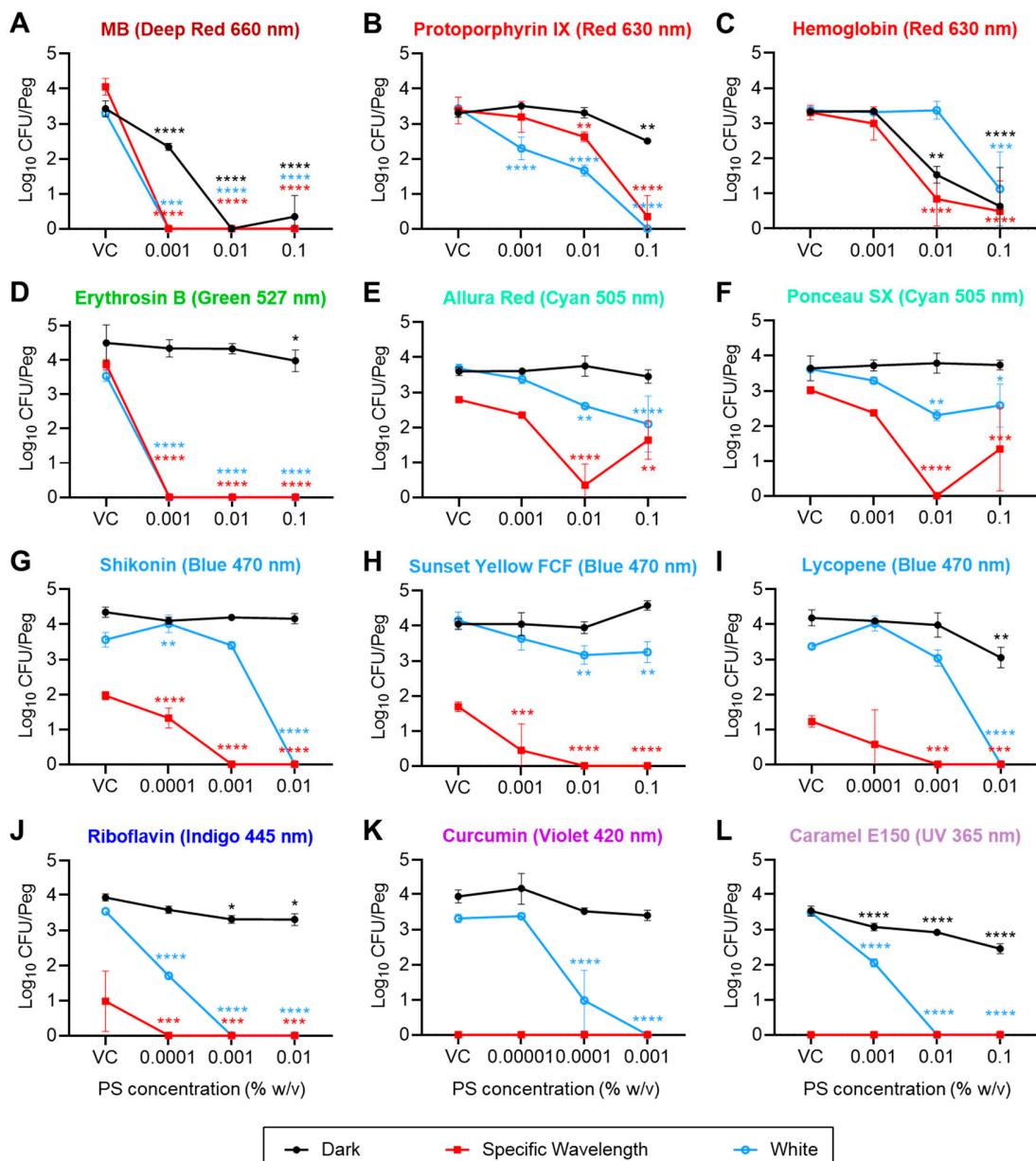


Figure S2. Recovery of viable *Pseudomonas fragi* ATCC 4973 from MBEC pegs (Log₁₀ CFU/peg). Biofilms were challenged with methylene blue (A), protoporphyrin IX (B), hemoglobin (C), erythrosin B (D), allura red (E), ponceau SX (F), shikonin (G), sunset yellow FCF (H), tomato lycopene extract (I), riboflavin (J), curcumin (K), or caramel color E150 (L), at the indicated concentrations (x-axis; VC = Vehicle Controls), and subjected to the indicated irradiation conditions: dark (represented by black lines) vs specific wavelength (monochromatic light indicated above each graph, represented by red lines) vs white light (polychromatic light represented by blue lines). Symbols and error bars represent the mean ± standard deviation for 3 replicate pegs. Statistical significance, as indicated for certain data points, was evaluated by 2-way ANOVA and p-values (relative to the corresponding "VC" data point for each irradiation condition) were corrected for multiple comparisons using Dunnett's method. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001.

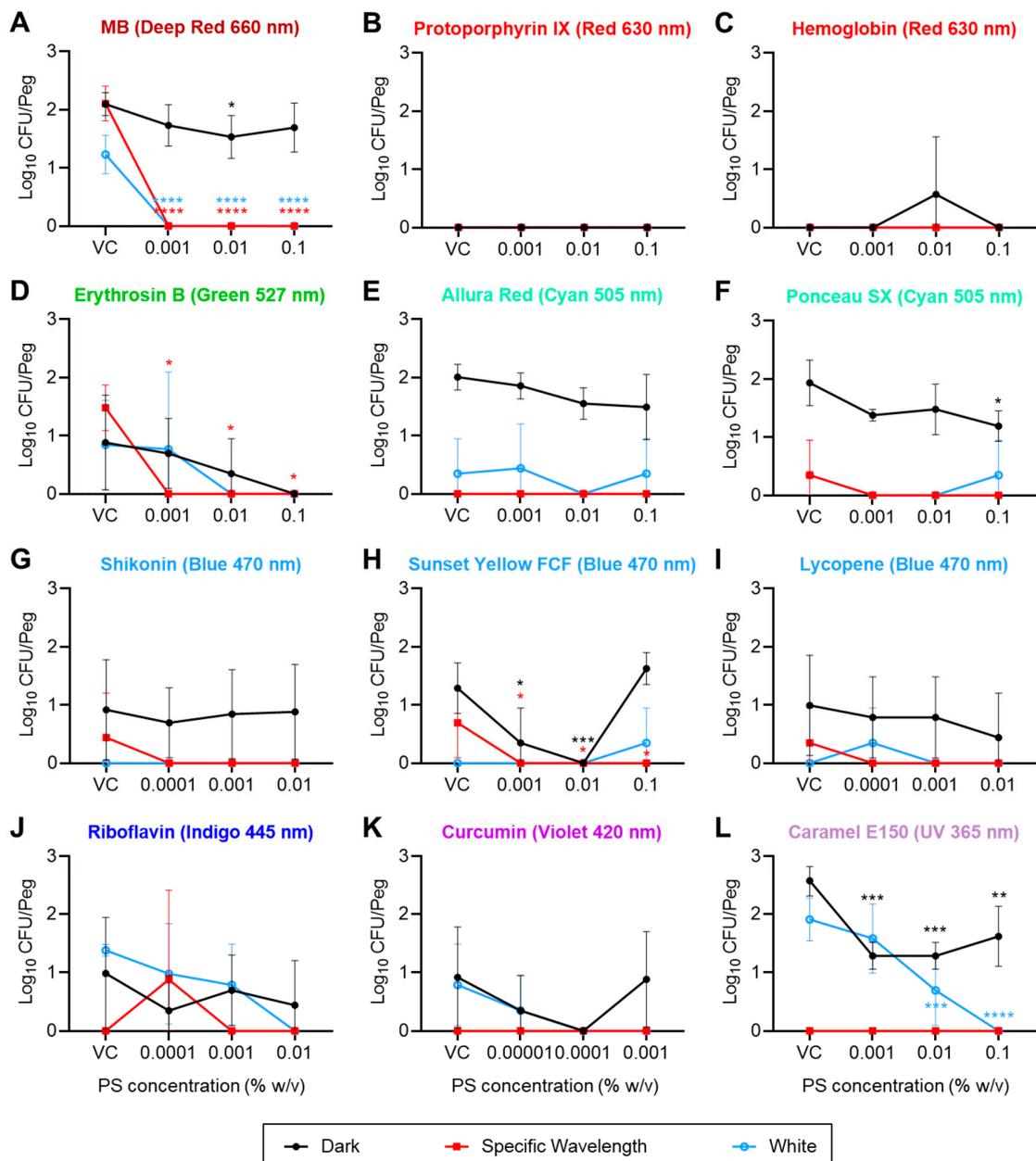


Figure S3. Recovery of viable *Brochothrix Thermosphacta* ATCC 11509 from MBEC pegs (Log₁₀ CFU/peg). Biofilms were challenged with methylene blue (A), protoporphyrin IX (B), hemoglobin (C), erythrosin B (D), allura red (E), ponceau SX (F), shikonin (G), sunset yellow FCF (H), tomato lycopene extract (I), riboflavin (J), curcumin (K), or caramel color E150 (L), at the indicated concentrations (x-axis; VC = Vehicle Controls), and subjected to the indicated irradiation conditions: dark (represented by black lines) vs specific wavelength (monochromatic light indicated above each graph, represented by red lines) vs white light (polychromatric light represented by blue lines). Symbols and error bars represent the mean \pm standard deviation for 3 replicate pegs. Statistical significance, as indicated for certain data points, was evaluated by 2-way ANOVA and *p*-values (relative to the corresponding "VC" data point for each irradiation condition) were corrected for multiple comparisons using Dunnett's method. *, *p*<0.05; **, *p*<0.01; ***, *p*<0.001; ****, *p*<0.0001.

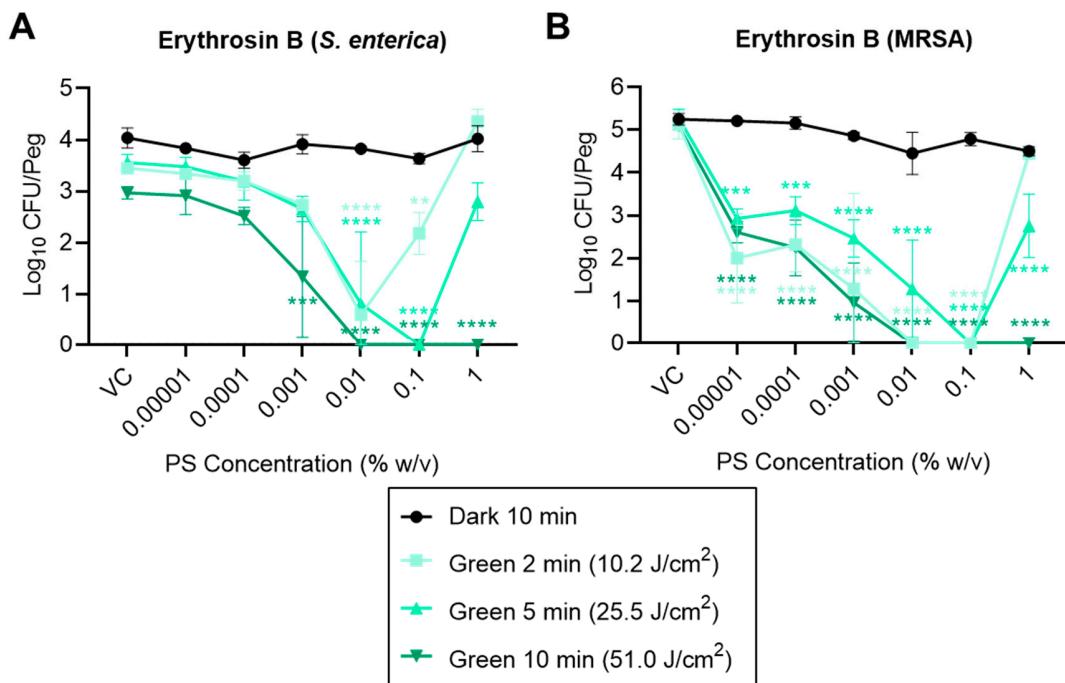


Figure S4. Recovery of viable *S. enterica* Choleraesuis ATCC 10708 (A) or methicillin resistant *Staphylococcus aureus* (MRSA 456 S. Yanke) (B) from green light-irradiated MBEC pegs (Log_{10} CFU/peg). Biofilms were challenged with erythrosin B, at the indicated concentrations (x-axis; VC = Vehicle Controls), for the indicated irradiation times. Symbols and error bars represent the mean \pm standard deviation for 3 replicate pegs. Statistical significance, as indicated for certain data points, was evaluated by 2-way ANOVA and p -values (relative to the corresponding “VC” data point for each irradiation condition) were corrected for multiple comparisons using Dunnett’s method. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

Table S1. Concentration of photosensitizer candidates used for screening.

Photosensitizer Candidate	Final Tested Concentrations (% w/v)	Final Tested Concentrations (mM)
Methylene Blue (Positive Control)	0.1, 0.01, 0.001	3.126, 0.313, 0.031
Protoporphyrin IX	0.1, 0.01, 0.001	1.777, 0.178, 0.018
Hemin	0.01, 0.001, 0.0001	0.153, 0.0153, 0.0015
Hemoglobin	0.1, 0.01, 0.001	0.0155, 0.0016, 0.0002
Titanium Dioxide^e	0.1, 0.01, 0.001	12.521, 1.252, 0.125
Orchil (Orcein)	0.01, 0.001, 0.0001	0.199, 0.019, 0.0019
Cochineal (Carmine)	0.01, 0.001, 0.0001	0.203, 0.0203, 0.002
Anthocyanin (Cyanidin-3-glucoside)	0.01, 0.001, 0.0001	0.206, 0.021, 0.0021
Betanin (Beet Red)	0.1, 0.01, 0.001	1.816, 0.182, 0.0018
Black Carrot Extract	0.1, 0.01, 0.001	-
Erythrosin B	0.1, 0.01, 0.001	1.136, 0.114, 0.0114
Acid Red 27 (Amaranth)	0.1, 0.01, 0.001	1.654, 0.165, 0.00165
Enocyanin (an Anthocyanin)	0.1, 0.01, 0.001	4.825, 0.482, 0.048
Resveratrol (Grape Skin Extract)	0.01, 0.001, 0.0001	0.438, 0.044, 0.0044
Citrus red No. 2	0.01, 0.001, 0.0001	0.324, 0.0324, 0.00324
Allura Red	0.1, 0.01, 0.001	2.014, 0.201, 0.0201
Ponceau SX	0.1, 0.01, 0.001	2.082, 0.208, 0.021
Canthaxanthin	0.001, 0.0001, 0.00001	0.018, 0.0018, 0.0002
Shikonin (Alkanet)	0.01, 0.001, 0.0001	0.347, 0.0347, 0.00347
Sunset Yellow FCF	0.1, 0.01, 0.001	2.2104, 0.221, 0.0221
Tomato Lycopene Extract	0.01, 0.001, 0.0001	0.186, 0.0186, 0.0019
Paprika (Capsanthin)	0.01, 0.001, 0.0001	0.038, 0.0038, 0.00038
Trans-β-apo-8'-carotenal	0.001, 0.0001, 0.00001	0.024, 0.0024, 0.00024
Saffron	0.1, 0.01, 0.001	1.024, 0.1024, 0.0102
Xanthophyll (Lutein)	0.01, 0.001, 0.0001	0.176, 0.0176, 0.0018
Ethyl β-apo-8'-carotenoate	0.001, 0.0001, 0.00001	0.022, 0.0022, 0.0022
Riboflavin	0.01, 0.001, 0.0001	0.266, 0.0266, 0.0027
Curcumin	0.001, 0.0001, 0.00001	0.027, 0.0027, 0.00027
Beta-Carotene	0.001, 0.0001, 0.00001	0.0186, 0.00186, 0.00019
Tartrazine (Acid Yellow 23)	0.1, 0.01, 0.001	1.871, 0.1871, 0.0187
Norbixin (Annatto)	0.01, 0.001, 0.0001	0.263, 0.0263, 0.0026
Turmeric Extract	0.001, 0.0001, 0.00001	-
Caramel E150	0.1, 0.01, 0.001	7.927, 0.793, 0.0793

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Table S2. Treatment conditions used for the optimization of the photosensitizer candidates. Treatment conditions used for food contact surfaces including aluminum, stainless steel, galvanized steel, mild steel, copper, and wood.

PS	λ (nm)	Concentration Range (%) w/v)	LED Irradiation Time	Dose (J/cm ²)
Treatment conditions for optimization of PS candidates				
Riboflavin-5'-Phosphate Sodium USP	470 (Blue)	1	5.0 min	0
		0.1	1.0 min	10.2
		0.01	2.5 min	25.5
		0.001	5.0 min	51.0
		0.0001		
		0.00001		
		VC=water		
Sunset Yellow		1		
		0.1		
		0.01		
		0.001		
		0.0001		
		0.00001		
		VC=water		
Curcumin		0.001		
		0.0001		
		0.00001		
		0.000001		
		0.0000001		
		0.00000001		
		VC=1% DMSO in water		
Erythrosin B	527 (Green)	1	10.0 min	
		0.1	2.0 min	
		0.01	5.0 min	
		0.001	10.0 min	
		0.0001		
		0.00001		
		VC=water		
Treatment conditions for food contact surfaces				
Riboflavin-5'-Phosphate	445 nm (Indigo)	0.1	5 min	55.5

Table S3. The minimum bactericidal concentration (MBC) and minimum biofilm eradication concentration (MBEC) values (in % w/v) of the less effective photosensitizers (based on the turbidimetric assay for *Salmonella enterica* Choleraesuis ATCC 10708, Methicillin Resistant *Staphylococcus aureus* (MRSA 456 S. Yanke), *Pseudomonas fragi* (ATCC 4973), and *Brochothrix Thermosphacta* (ATCC 11509).

Photosensitizer	LED	Turbidimetric Assay							
		<i>S. enterica</i>		MRSA		<i>P. fragi</i>		<i>B. thermosphacta</i>	
		MBC	MBEC	MBC	MBEC	MBC	MBEC	MBC	MBEC
Protoporphyrin IX	Dark	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	N/A	N/A
	Red	>0.1	>0.1	>0.1	>0.1	>0.1	0.1?	N/A	N/A
	White	0.1?	>0.1	0.01	0.01	0.1	0.1	N/A	N/A
Hemin	Dark	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	N/A	N/A
	Red	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	N/A	N/A
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	N/A	N/A
Titanium dioxide	Dark	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	Amber	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	White	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
Orcein (Orchil)	Dark	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	Amber	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	0.01?	0.01?
Carmine (Cochineal)	Dark	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	Amber	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	0.01	0.001
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
Cyanidin-3-O-glucoside	Dark	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	Green	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
Betanin	Dark	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	Green	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	White	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
Black Carrot Extract	Dark	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	Green	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	White	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
Acid Red 27 (Amaranth)	Dark	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	Green	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	0.01	0.01
	White	0.1?	0.01?	>0.1	>0.1	0.1?	0.01	0.01	0.01
Enocyanin	Dark	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	0.1?	>0.1
	Green	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	0.1	0.1?
	White	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	0.1	0.1?
Resveratrol	Dark	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	Green	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
Citrus Red No. 2	Dark	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	Cyan	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	N/A
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
Canthaxanthin	Dark	>0.01	>0.01	>0.01	>0.01	>0.001	>0.001	>0.001	>0.001
	Blue	>0.01	>0.01	>0.01	>0.01	>0.001	>0.001	>0.001	>0.001
	White	>0.01	>0.01	>0.01	>0.01	>0.001	>0.001	N/A	>0.001
Capsanthin	Dark	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	Blue	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	0.01?	N/A
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	N/A

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Trans-β-apo-8'-carotenal	Dark	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001
	Blue	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	0.0001?	N/A
	White	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	0.001?
Saffron	Dark	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	Blue	>0.1	>0.1	>0.1	>0.1	0.1?	0.1	0.1?	N/A
	White	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	0.01
Lutein	Dark	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
	Indigo	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	N/A
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
Ethyl β-apo-8'-carotenoate	Dark	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001
	Indigo	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	N/A
	White	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001
β-carotene	Dark	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	0.001?	0.001
	Violet	0.0001?	0.0001	>0.001	>0.001	>0.002	N/A	N/A	0.001?
	White	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	N/A
Acid Yellow 23	Dark	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	0.1?	N/A
	Violet	0.001*	N/A	0.01	0.01	0.001?	N/A	N/A	0.1
	White	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	N/A
Norbixin	Dark	>0.01	>0.01	0.01	>0.01	>0.01	>0.01	0.01	N/A
	Violet	0.001*	0.001*	0.01	0.001	0.001	N/A	N/A	N/A
	White	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	0.01	N/A
Turmeric extract	Dark	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	0.001?	0.001?
	Violet	N/A	0.00001*	>0.001	>0.001	0.001?	N/A	N/A	>0.001
	White	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	N/A

? = 2 of 3 wells clear

N/A = No growth in at least 2 of 3 VC wells

* = only 2 of 3 VC wells grew

Max = "Maximum kill" (zero CFUs recovered from the treated wells; magnitude of the log reduction is determined by the recovery from the VC wells).

For the quantitative data, green highlighting indicates *p* values <0.05 (based on an unpaired, two-tailed T-test, relative to the appropriate vehicle control) or \log_{10} CFU/peg reductions >1.

Table S4. The minimum bactericidal concentration (MBC) and minimum biofilm eradication concentration (MBEC) values (in % w/v) of riboflavin and riboflavin-5'-phosphate (based on the turbidimetric and quantitative assay) for MRSA and *Salmonella enterica* Choleraesuis (ATCC 10708). Recovery of viable MRSA and *S. enterica* from indigo irradiated MBEC pegs (\log_{10} CFU/peg) are also included. Biofilms were challenged with riboflavin and riboflavin-5'-phosphate at the indicated concentrations, for 5 min (corresponding to 55.5 J/cm^2 dose) with 445 nm LED light (n=3).

PS	Organism	Turbidimetric Assay		Quantitative assay	
		MBC (% w/v)	MBEC (% w/v)	Quant. MBEC (% w/v)	\log_{10} CFU reduction ^a
Riboflavin	MRSA	>0.01	>0.01	>0.01	1.76±0.43
	<i>S. enterica</i>	>0.01	>0.01	>0.01	1.04±1.31
Riboflavin-5'-Phosphate	MRSA	0.01	0.01	0.01	4.17 ^{max} ±0.00
	<i>S. enterica</i>	0.1	0.1	0.1	3.05 ^{max} ±0.00

^aValues in this column are given as mean ± SD (n=3).

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Table S5. The minimum bactericidal concentration (MBC) and minimum biofilm eradication concentration (MBEC) values (in % w/v) of the selected photosensitizers (based on the turbidimetric and quantitative assay) for *Salmonella enterica* Choleraesuis (ATCC 10708).

PS	Dose (J/cm ²)	Turbidimetric Assay		Quant. MBEC (% w/v)	Notes
		MBC (% w/v)	MBEC (% w/v)		
Riboflavin- 5'- Phosphate	0 (Dark)	>1	>1	>1	
	10.2	>1	>1	>1	
	25.5	>1	>1	>1	
	51.0	0.1	0.01	0.1	
Sunset Yellow	0 (Dark)	>1	>1	>1	
	10.2	>1	>1	>1	
	25.5	>1	>1	>1	
	51.0	0.01	0.001	0.001	
Curcumin	0 (Dark)	>0.001	>0.001	>0.001	
	10.2	>0.001	>0.001	>0.001	
	25.5	>0.001	>0.001	>0.001	
	51.0	>0.001	>0.001	0.0001	
Riboflavin- 5'- Phosphate + Sunset Yellow	0 (Dark)	>1, >1	>1, >1	>1, >1	Antagonistic effect: Sunset Yellow lost potency relative to individual.
	10.2	>1, >1	>1, >1	>1, >1	
	25.5	>1, >1	>1, >1	>1, >1	
	51.0	0.01, 0.01	0.01, 0.01	0.01, 0.01	
Sunset Yellow + Curcumin	0 (Dark)	>1, >0.001	>1, >0.001	>1, >0.001	Neutral: Sunset Yellow same potency as individual; Curcumin did not hinder.
	10.2	>1, >0.001	>1, >0.001	>1, >0.001	
	25.5	>1, >0.001	>1, >0.001	>1, >0.001	
	51.0	0.001, 0.000001	0.001, 0.000001	0.001, 0.000001	
Riboflavin- 5'- Phosphate + Curcumin	0 (Dark)	>1, >0.001	>1, >0.001	>1, >0.001	Synergistic effect: Improved efficacy at 2.5 min = synergy. Improved efficacy at 0.01% R-5-P, 0.00001% Curcumin = Synergy.
	10.2	>1, >0.001	>1, >0.001	>1, >0.001	
	25.5	0.1, 0.0001	0.1, 0.0001	0.1, 0.0001	
	51.0	0.1, 0.0001	0.1, 0.0001	0.01, 0.00001	
Erythrosin B	0 (Dark)	>1	>1	>1	Erythrosin B is potent, even at 25.5 J/cm ² .
	10.2	0.01	0.01	>1	
	25.5	0.01	0.01	0.1	
	51.0	0.01	0.01	0.01	

Note: For *S. enterica*, "Quant. MBEC" = lowest concentration yielding either $>3 \log_{10}$ reduction OR max kill (whichever is greater). For turbidimetric data, yellow shading indicates that 3 of 4 wells were clear ("Blue" set) or 2 of 3 wells were clear (Erythrosin B).

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Table S6. The minimum bactericidal concentration (MBC) and minimum biofilm eradication concentration (MBEC) values (in % w/v) of the selected photosensitzers (based on the turbidimetric and quantitative assay) for Methicillin resistant *Staphylococcus aureus* (MRSA 456 S. Yanke).

PS	Dose (J/cm ²)	Turbidimetric Assay		Quant. MBEC (% w/v)	Notes
		MBC (% w/v)	MBEC (% w/v)		
Riboflavin-5'-Phosphate	0 (Dark)	>1	>1	>1	
	10.2	>1	>1	>1	
	25.5	0.1	>1	>1	
	51.0	0.1	0.1	0.1	
Sunset Yellow	0 (Dark)	>1	>1	>1	
	10.2	>1	>1	>1	
	25.5	>1	>1	>1	
	51.0	0.01	0.01	0.01	
Curcumin	0 (Dark)	>0.001	>0.001	>0.001	
	10.2	>0.001	>0.001	>0.001	
	25.5	>0.001	>0.001	>0.001	
	51.0	0.001	>0.001	0.001	
Riboflavin-5'-Phosphate + Sunset Yellow	0 (Dark)	>1, >1	>1, >1	>1, >1	Neutral: Sunset Yellow same potency as individual; R-5-P did not hinder.
	10.2	>1, >1	>1, >1	>1, >1	
	25.5	>1, >1	>1, >1	>1, >1	
	51.0	0.01, 0.01	0.01, 0.01	0.01, 0.01	
Sunset Yellow + Curcumin	0 (Dark)	>1, >0.001	>1, >0.001	>1, >0.001	Neutral: Sunset Yellow same potency as individual; Curcumin did not hinder.
	10.2	>1, >0.001	>1, >0.001	>1, >0.001	
	25.5	>1, >0.001	>1, >0.001	>1, >0.001	
	51.0	0.01, 0.00001	0.01, 0.00001	0.01, 0.00001	
Riboflavin-5'-Phosphate + Curcumin	0 (Dark)	>1, >0.001	>1, >0.001	>1, >0.001	Neutral: R-5-P same potency as individual; Curcumin did not hinder.
	10.2	0.1, 0.0001	>1, >0.001	>1, >0.001	
	25.5	0.1, 0.0001	>1, >0.001	>1, >0.001	
	51.0	0.1, 0.0001	0.1, 0.0001	0.1, 0.0001	
Erythrosin B	0 (Dark)	1	>1	>1	Erythrosin B is potent, even at 10.2 J/cm ² .
	10.2	0.001	0.1	0.01	
	25.5	0.0001	0.1	0.01	
	51.0	0.0001	0.01	0.001	

Note: For MRSA, "Quant. MBEC" = lowest concentration yielding >4 log₁₀ reduction. For turbidimetric data, yellow shading indicates that 3 of 4 wells were clear ("Blue" set).