

**Electronic Supplementary Information** for the  
article: “*Podoviridae* bacteriophage for the  
biocontrol of *Pseudomonas aeruginosa* in  
rainwater.”

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### ***Legionella* spp. growth conditions and host range determination**

*Legionella* spp. were grown at 35 °C for up to 4 days on buffered charcoal yeast extract (BCYE) agar (Oxoid, LTD, Hampshire, England) supplemented with *Legionella* BCYE growth supplement [buffer/potassium hydroxide (10 g/L), ferric pyrophosphate (0.25 g/L), alpha-ketoglutarate (1.0 g/L) and L-cysteine-HCl (0.4 g/L); Oxoid]. For liquid cultures, *Legionella* spp. were inoculated into Lennox broth [10 g/L Tryptone (Biolab, Merck, Wadeville, South Africa), 5 g/L Yeast Extract (Biolab, Merck), 5 g/L sodium chloride (NaCl; Saarchem, Durban, South Africa)] supplemented with *Legionella* BCYE growth supplement (Oxoid) and incubated at 37 °C for 48 hrs on a test tube rotator (New Brunswick Scientific, NY, USA).

In order to assess whether the isolated bacteriophages displayed activity against *Legionella* spp., host range determination was performed using spot tests as explained in the main manuscript file. In order to increase the visibility of potential plaques (lytic zones), the soft top-agar layer which was inoculated with the *Legionella* strain to be tested (normally BCYE agar), consisted of Lennox Broth supplemented with 0.6% agar bacteriological and *Legionella* BCYE growth supplement (Oxoid), while the bottom layer was conventional BCYE agar (Oxoid).

### **Nucleic acid analysis of PFW25**

In contrast, the genome size of PFW25 could not accurately be assessed as the DNA from PFW25 was not digested by *EcoRI* or *ClaI*. It has been reported that both *EcoRI* and *ClaI* are sensitive to prokaryotic DNA methylation,<sup>1</sup> thus the inability of these restriction enzymes to cleave the DNA from PFW25 may possibly be attributed to DNA methylation of the PFW25 genome. As reported by Labrie *et al.* methylated bacteriophage DNA are insensitive to cognate restriction enzymes and may therefore survive the restriction-modification systems employed by bacteria to prevent bacteriophage infection.<sup>2</sup> This resistance may be achieved through the acquisition of the cognate methylase gene in the bacteriophage genome.<sup>2</sup> Additionally, an anti-restriction system has been identified in bacteriophage T4 (*Myoviridae*), where the bacteriophage genome does not contain cytosine, but rather hydroxymethylcytosine, making bacteriophage T4 DNA impervious to restriction modification systems that recognise sequences containing cytosine.<sup>2</sup>

**Table S1** Target and non-target bacterial species utilised during the host range determination experiments.

Organism	Classification (Source)	Reference
<b><u>Target bacterial species</u></b>		
<i>Pseudomonas aeruginosa</i> ATCC 27853	Reference	ATCC
<i>Pseudomonas aeruginosa</i> ATCC 333339	Reference	ATCC
<i>Pseudomonas aeruginosa</i> PAO1	Clinical*	3
<i>Pseudomonas aeruginosa</i> I12	Environmental (Rainwater)	KCC
<i>Pseudomonas aeruginosa</i> Y12	Environmental (Rainwater)	KCC
<i>Pseudomonas aeruginosa</i> ST5	Environmental (Wastewater)	KCC <sup>4</sup>
<i>Pseudomonas aeruginosa</i> S2 37	Environmental (Pasteurized Rainwater - 72 °C)	KCC <sup>5</sup>
<i>Pseudomonas aeruginosa</i> S1 68	Environmental (Pasteurized Rainwater - 70 °C)	KCC <sup>5</sup>
<i>Pseudomonas aeruginosa</i> S4 12	Environmental (Pasteurized Rainwater - 89 °C)	KCC <sup>5</sup>
<i>Pseudomonas aeruginosa</i> S1 106	Environmental (Pasteurized Rainwater - 70 °C)	KCC <sup>5</sup>
<i>Pseudomonas aeruginosa</i> T1 (PA3)	Clinical*	KCC <sup>6</sup>
<i>Pseudomonas aeruginosa</i> MCC3 (PA2)	Clinical*	KCC <sup>6</sup>
<i>Pseudomonas fluorescens</i> ATCC 13525	Reference	ATCC
<i>Pseudomonas fluorescens</i> C512	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Pseudomonas fluorescens</i> 6B	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Pseudomonas fluorescens</i> 6K	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Pseudomonas protegens</i> ATCC 17386	Reference	ATCC
<i>Pseudomonas putida</i> 10	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Pseudomonas rhodesiae</i> C58	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Pseudomonas syringae</i> 7B	Environmental (Rainwater)	KCC <sup>7</sup>
<b><u>Non-target bacterial species</u></b>		
<i>Acinetobacter baumannii</i> ATCC 19606	Reference	ATCC
<i>Acinetobacter baumannii</i> MCC1 (AB2)	Clinical*	KCC <sup>6</sup>
<i>Acinetobacter baumannii</i> T1 (AB3)	Clinical*	KCC <sup>6</sup>

**Table S1 (continued)** Target and non-target bacterial species utilised during the host range determination experiments.

Organism	Classification (Source)	Reference
<b><u>Non-target bacterial species</u></b>		
<i>Acinetobacter calcoaceticus</i> C55	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Aeromonas hydrophila</i> ATCC 35654	Reference	ATCC
<i>Bacillus cereus</i> S1 77	Environmental (Rainwater)	KCC <sup>5</sup>
<i>Bacillus subtilis</i> ATCC 6051	Reference	ATCC
<i>Bacillus subtilis</i> ATCC 6633	Reference	ATCC
<i>Enterobacter aerogenes</i> ATCC 13048	Reference	ATCC
<i>Enterobacter cloacae</i> 3T	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Enterobacter ludwigii</i> 6I	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Escherichia coli</i> ATCC 13706	Reference	ATCC
<i>Escherichia coli</i> ATCC 417561	Reference	ATCC
<i>Escherichia coli</i> MCC2 (EC2)	Clinical*	KCC <sup>6</sup>
<i>Escherichia coli</i> S7 5	Environmental (Rainwater)	KCC <sup>5</sup>
<i>Escherichia coli</i> S5 56	Environmental (Rainwater)	KCC <sup>5</sup>
<i>Escherichia coli</i> S7 13	Environmental (Rainwater)	KCC <sup>5</sup>
<i>Escherichia coli</i> T1 (EC3)	Clinical*	KCC <sup>6</sup>
Enteroaggregative <i>Escherichia coli</i> ATCC 3591-87	Reference	ATCC
Enterohemorrhagic <i>Escherichia coli</i> (O157:H7)	Reference	ATCC
<i>Enterococcus faecalis</i> CAB 176 (S1)	Clinical*	KCC <sup>8</sup>
<i>Enterococcus faecalis</i> C513	Environmental (Rainwater)	KCC
<i>Enterococcus faecium</i> 8D	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Enterococcus faecium</i> Clin	Clinical*	KCC
<i>Klebsiella pneumoniae</i> ATCC 333305	Reference	ATCC
<i>Klebsiella pneumoniae</i> ATCC 10031	Reference	ATCC
<i>Klebsiella pneumoniae</i> ATCC 13893	Reference	ATCC

**Table S1 (continued)** Target and non-target bacterial species utilised during the host range determination experiments.

Organism	Classification (Source)	Reference
<b><u>Non-target bacterial species</u></b>		
<i>Klebsiella pneumoniae</i> ATCC 417503	Reference	ATCC
<i>Klebsiella pneumoniae</i> S1 43	Environmental (Pasteurized Rainwater - 70 °C)	KCC <sup>5</sup>
<i>Klebsiella pneumoniae</i> ST 23	Environmental (Wastewater)	KCC <sup>4</sup>
<i>Klebsiella pneumoniae</i> ST 15	Environmental (Wastewater)	KCC <sup>4</sup>
<i>Klebsiella pneumoniae</i> MCC3 (KP2)	Clinical*	KCC <sup>6</sup>
<i>Klebsiella pneumoniae</i> P2	Clinical*	KCC <sup>8</sup>
<i>Klebsiella pneumoniae</i> P3	Clinical*	KCC <sup>8</sup>
<i>Klebsiella oxytoca</i> 2A	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Klebsiella oxytoca</i> 2E	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Legionella longbeachae</i> ATCC 33462	Reference	ATCC
<i>Legionella longbeachae</i> 45 L1	Environmental (Rainwater)	KCC <sup>9</sup>
<i>Legionella longbeachae</i> 45 L13	Environmental (Rainwater)	KCC <sup>9</sup>
<i>Legionella pneumophila</i> ATCC 33152	Reference	ATCC
<i>Legionella norrlandica</i> 45 L10	Environmental (Rainwater)	KCC <sup>9</sup>
<i>Legionella norrlandica</i> 45 L18	Environmental (Rainwater)	KCC <sup>9</sup>
<i>Listeria monocytogenes</i> 412P	Environmental (Food - Pork)	BIOPEP <sup>10</sup>
<i>Listeria ivanovii</i> ATCC 19119	Reference	ATCC
<i>Salmonella typhimurium</i> ATCC 14028	Reference	ATCC
<i>Serratia fonticola</i> 6E	Environmental (Rainwater)	KCC <sup>7</sup>
<i>Serratia marcescens</i> ATCC 13880	Reference	ATCC
<i>Shigella sonnei</i> ATCC 25931	Reference	ATCC
<i>Shigella sonnei</i> C6C	Environmental (Rainwater)	KCC
<i>Staphylococcus aureus</i> ATCC 25923	Reference	ATCC
<i>Staphylococcus aureus</i> ATCC 333292	Reference	ATCC

**Table S1 (continued)** Target and non-target bacterial species utilised during the host range determination experiments.

Organism	Classification (Source)	Reference
<b><u>Non-target bacterial species</u></b>		
<i>Staphylococcus equorum</i> Sp2	Environmental (River)	KCC
<i>Staphylococcus saprophyticus</i> 2.2S1	Environmental (Fresh Rainwater)	KCC
Methicillin-resistant <i>Staphylococcus aureus</i> XEN36	Clinical*	KCC <sup>8</sup>
<i>Yersinia enterocolitica</i> ATCC 27729	Reference	ATCC
<i>Yersinia rohdei</i> 21	Environmental (Rainwater)	KCC
<i>Yersinia rohdei</i> L6D	Environmental (Rainwater)	KCC

ATCC – American Type Culture Collection; KCC – Khan Laboratory Culture Collection (Department of Microbiology, Stellenbosch University); BIOPEP - BIOPEP Peptide Group (Department of Biochemistry, Stellenbosch University); DFS-SU - Department of Food Science (Stellenbosch University)

\*Clinical isolates were obtained from the KCC but were originally sourced from either the Department of Biomedical Sciences at Cape Peninsula University of Technology or the Department of Pathology (Division of Medical Microbiology) at Stellenbosch University.

**Table S2** Results obtained using the Podo-Hypo-F/R (*Podoviridae*) and Myo-Hypo-F/R (*Myoviridae*) primer sets.

Bacteriophage	Primer Set	Similar Bacteriophage (NCBI accession no.)	Sequence Identity	Query Coverage	Reference
PAW33	Podo-Hypo-F/R	LP14 (MH356729.1)	83%	92%	Unpublished (NCBI only)
		YH30 (KP994390.1)	85%	82%	11
		phi176 (KM411960.1)	85%	82%	Unpublished (NCBI only)
		Pa2 (NC_027345.1)	85%	82%	Unpublished (NCBI only)
PFW25	Myo-Hypo-F/R	vB_Kpn_F48 (MG746602.1)	99%	99%	12

**Table S3** qPCR performance characteristics obtained for the absolute quantification of *P. aeruginosa* S1 68 and PAW33 and relative quantification of the *rpoS*, *phzM*, *RecA* and *LexA* genes in the 8 and 24 hr pre-treatment followed by SODIS trial

Organism/Gene	Primer Set	qPCR Efficiency	Slope	Y-intercept	LLOD	Melting Peak
<i>P. aeruginosa</i>	PS1/PS2	1.84 to 1.94 (92% to 97%)	-3.7821 to -3.4677	34.90 to 38.00	2 to 9 GC/μL	89.20 °C (±1 °C)
PAW33	Podo-Hypo-F/R	1.98 to 2.06 (99% to 103%)	-3.366 to -3.1865	33.50 to 34.38	5 to 12 GC/μL	84.25 °C (±1 °C)
<i>rpoS</i>	rpoS-F/R	1.81 to 1.96 (90.5% to 98%)	-3.8675 to -3.4192	33.53 to 38.04	3 to 16 GC/μL	87.75 °C (±1 °C)
<i>phzM</i>	phzM-F/R	1.91 to 2.04 (95.5% to 102%)	-3.5675 to -3.2309	34.43 to 35.16	2 to 12 GC/μL	89 °C (±1 °C)
<i>recA</i>	RECA-RT1/RT2	2.09 (104.5%)	-3.1300 to -3.1217	35.35 to 36.80	3 to 6 GC/μL	90 °C (±1 °C)
<i>lexA</i>	LEXA-RT1/RT2	1.91 to 1.94 (95.5% to 97%)	-3.7158 to -3.5651	38.88 to 40.14	3 to 8 GC/μL	86 °C (±1 °C)

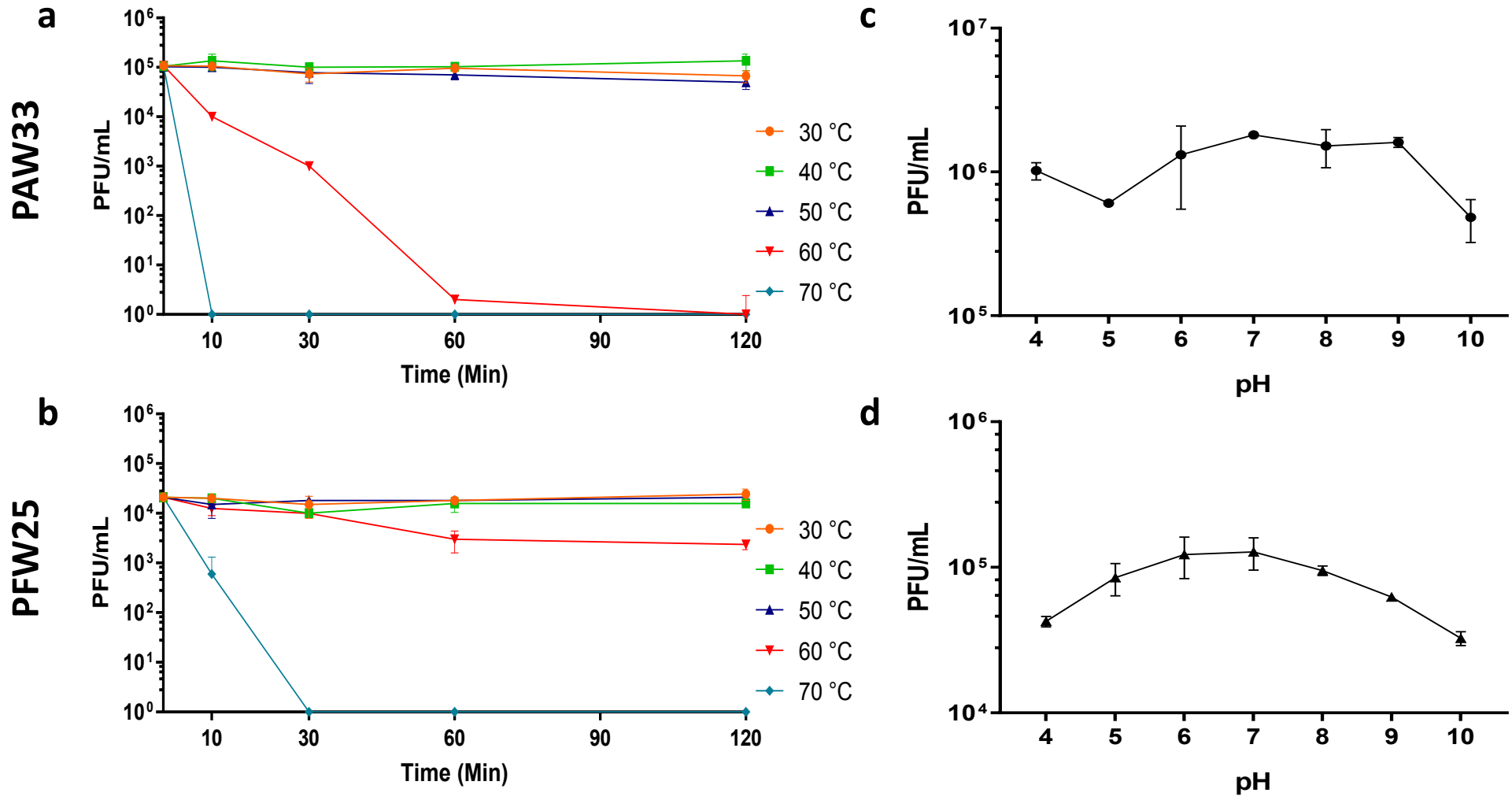


**Table S4** Summary of the cell counts, gene copies and log reductions obtained during the 8 hr and 24 hr trial for *P. aeruginosa* S1 68 and PAW33

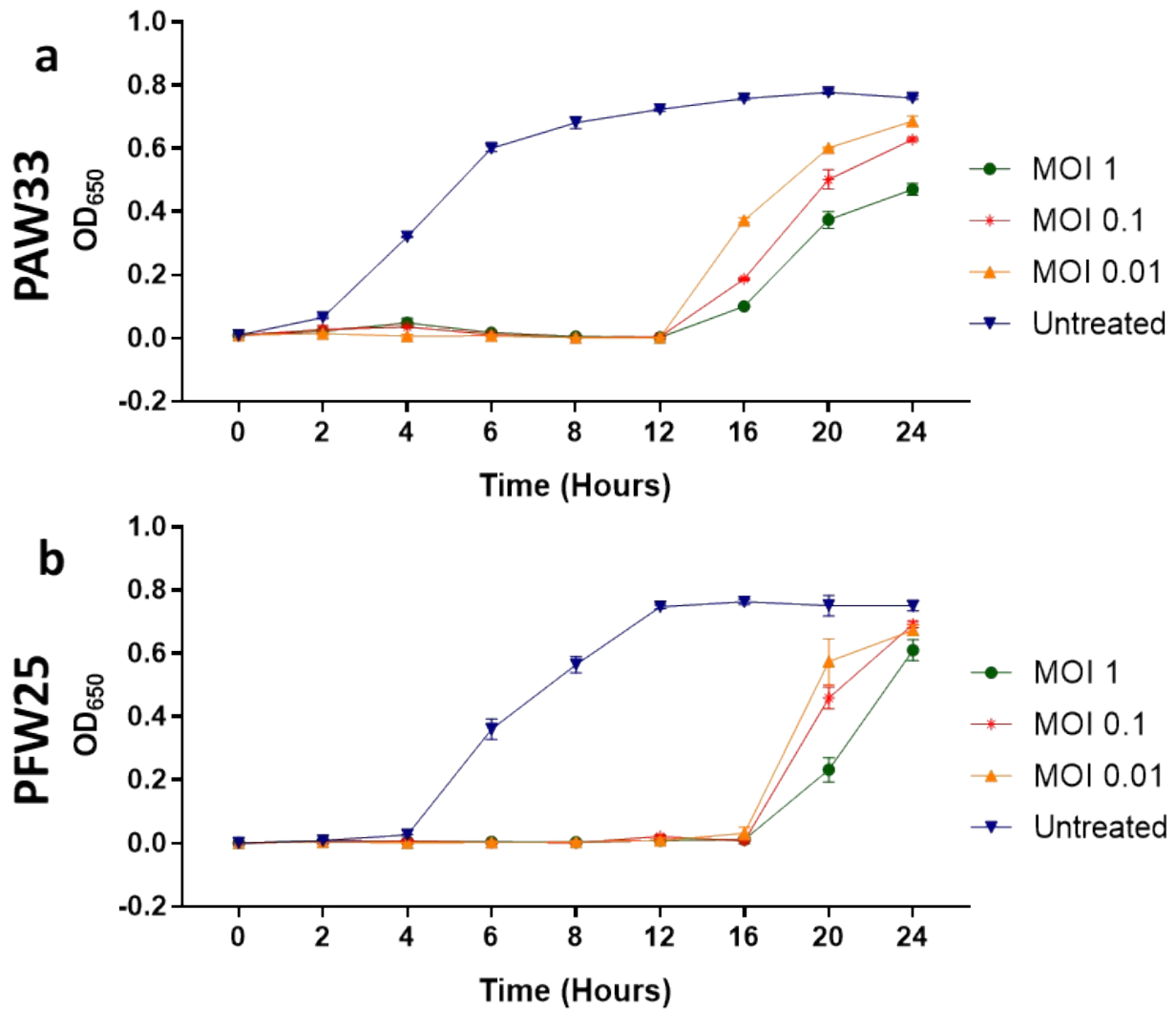
Trial	Sample Composition	Cell Counts (CFU or PFU/mL)			Viability qPCR (GC/mL)		
		Initial* Concentration	Final** Concentration	Log Reduction	Initial* Concentration	Final** Concentration	Log Reduction
8 hr Trial	Pre-treated <i>P. aeruginosa</i> S1 68	$1.24 \times 10^7$	$2.58 \times 10^3$	3.68	$6.98 \times 10^5$	$3.15 \times 10^3$	2.34
	PAW33	$6.00 \times 10^4$	$1.20 \times 10^2$	2.70	$1.80 \times 10^4$	$1.42 \times 10^4$	0.12
	Non-pre-treated ( <i>P. aeruginosa</i> S1 68)	$1.38 \times 10^7$	$2.50 \times 10^3$	3.74	$6.13 \times 10^5$	$2.80 \times 10^3$	2.33
24 hr Trial	Pre-treated <i>P. aeruginosa</i> S1 68	$2.03 \times 10^7$	$5.00 \times 10^2$	4.61	$2.23 \times 10^7$	$1.07 \times 10^4$	2.32
	PAW33	$8.00 \times 10^4$	$1.30 \times 10^2$	2.79	$2.50 \times 10^4$	$4.80 \times 10^3$	0.72
	Non-pre-treated ( <i>P. aeruginosa</i> S1 68)	$2.08 \times 10^7$	$2.50 \times 10^3$	3.91	$2.71 \times 10^6$	$1.47 \times 10^4$	2.26

\* Concentration at the start of the pre-treatment trial

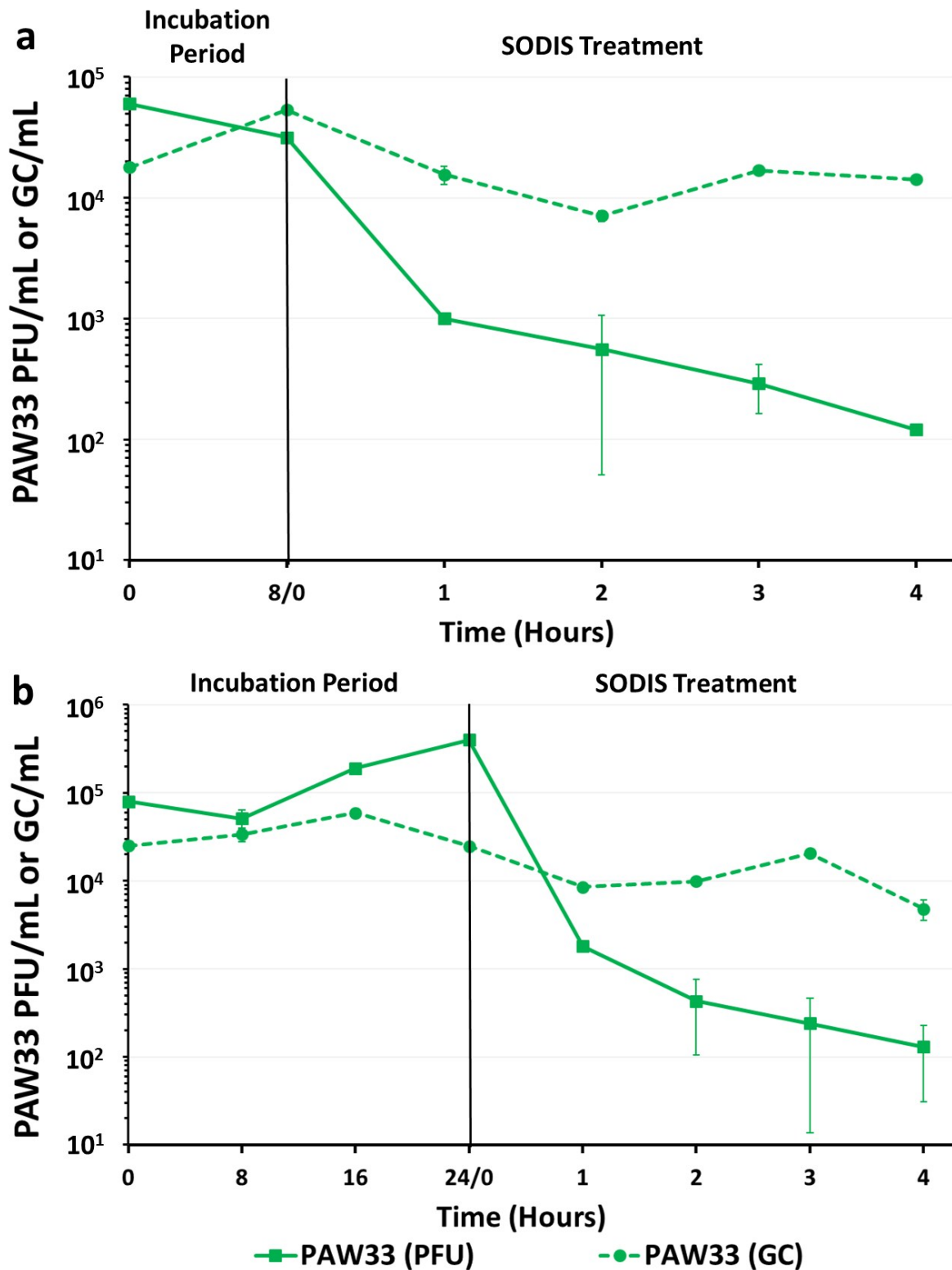
\*\* Concentration following the 4 hr SODIS-CPC treatment



**Fig. S1** Temperature sensitivity of (a) PAW33 and (b) PFW25 and pH sensitivity of (c) PAW33 and (d) PFW25. The data points represent the means of duplicate samples, the error bars indicate standard deviation (SD).



**Fig. S2** Optical density readings (at 650 nm) of (a) *P. aeruginosa* ATCC 27853 in co-culture with PAW33 and (b) *K. pneumoniae* ATCC 10031 in co-culture with PFW25. Samples were treated at different MOI's [1 = ●; 0.1 = ✕ and 0.01 = ▲] and an untreated control (▼; only target host) was included for each assay. The data points represent the means of triplicate samples, the error bars indicate standard deviation (SD).



**Fig. S3** PAW33 PFU/mL (■) and GC/mL (●) in the “PAW33 Pre-treatment” sterile rainwater sample during the (a) 8 hr pre-treatment and (b) 24 hr pre-treatment followed by the 4 hr SODIS-CPC disinfection. The data points represent the means of duplicate samples, the error bars indicate standard deviation (SD).

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