Supplementary Information: Glutaraldehyde resistance mechanisms in *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* biofilms

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Figure S1. Effect of glutaraldehyde exposure on *P. fluorescens* biofilm formation as measured by crystal violet. *P. fluorescens* biofilms grown to 24h or 72h were washed three times and stained with 0.3 % crystal violet for 20 min. The stain was washed and dissolved with 200 µl of DMSO and plates were read at 570 nm. Mean ±SD of three biological replicates are presented.

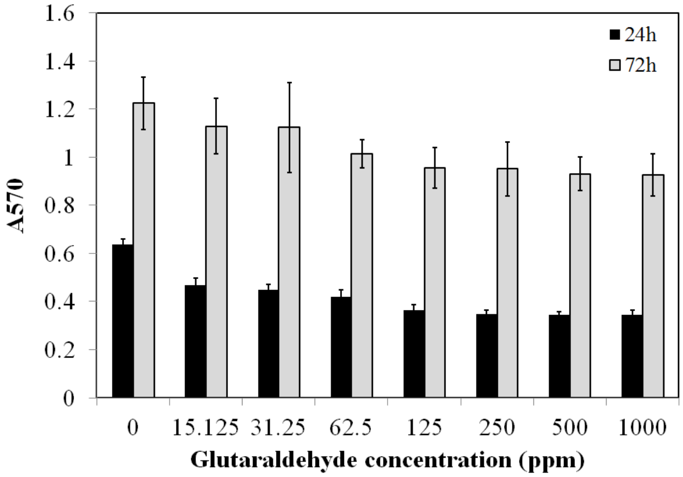

Figure S2: Effect of glutaraldehyde on *P. fluorescens* biofilm viability. *P. fluorescens* biofilms were grown for 72 h and washed three times and treated with indicated concentrations of glutaraldehyde for 10 min. Following exposure, the biofilms were washed, briefly sonicated to disperse the cells and serially diluted in PBS. The serial dilutions were plated on cetrimide agar plates and incubated at 25 °C. The colonies were counted after 24 h and presented as cfu/ml. Star denotes significant difference from control (PBS).

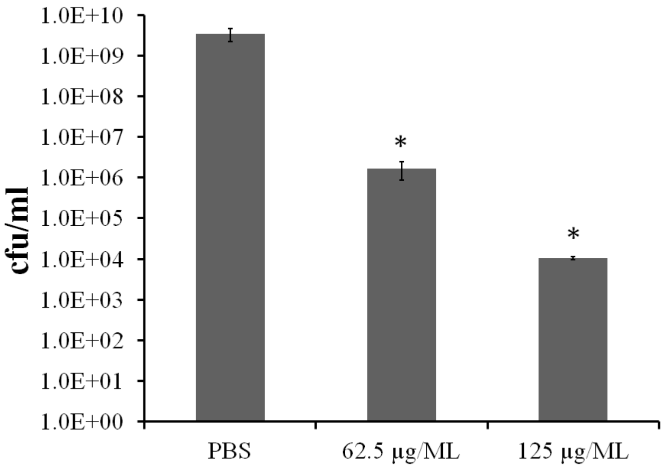

Table S1. Sequence of the primers used in the study to amplify selected genes using qPCR

Gene	Forward primer	Reverse primer
rpoD	CTGATCCAGGAAGGCAACAT	TGAGCTTGTTGATCGTCTCG
PFLU2929	CGTCGAGCAGTACCAGGAAG	GAGAACGCCTTCAGCGACTA
PFLU3876	ATGCCATCGACGTTGCTGTA	GCAACGTGGTGGAAATCAC
gyrA	CTCCCGGTCAATATCGAAGA	GATCACGTCACCGACAACAC

Table S2: Summary of RNA sequencing run.

	Replicate 1		Replicate 2	
Sample	PBS	Glut	PBS	Glut
Data generated (GB)	1.49	1.35	1.18	1.40
Total read count (million)	8.03	7.28	5.89	7.34
Percent reads aligned to coding sequences	70.52	70.87	73.44	72.42
Percent reads mapped to intergenic region	29.48	29.13	26.56	27.58
Average coverage	65.88	62.78	70.32	66.85

PBS = Phosphate Buffered Saline exposed biofilm; Glut= Glutaraldehyde exposed biofilm

Table S3: List of differentially regulated genes in *Pseudomonas fluorescens* SBW25 upon 62.5 mg/L glutaraldehyde exposure. The differentially regulated genes were determined through RNA-seq experiment. The raw reads were exported into CLC Genomics workbench 6.5.1, trimmed and mapped to *P. fluorescens* SBW25 genome (Genbank accession NC_012660). RPKM values were calculated after depletion of reads mapping to 16s and data was further normalized using scaling. Differentially expressed genes were identified using Baggerly's test on proportions.

Gene Name	Gene title	Fold change	p-value (FDR correction)
Amino acid transport and metabolism			
PFLU4644	putative racemase	2.08	0.047
PFLU0811	putative ornithine decarboxylase	2.23	0.003
PFLU2793	putative aminotransferase	2.28	0.004
PFLU0293	agmatine deiminase	2.29	0.000
PFLU1724	hypothetical protein	2.66	0.010
PFLU4022	putative allophanate hydrolase subunit 2	2.84	0.043
PFLU5634	putative amino acid ABC transporter membrane protein	3.00	0.005
Carbohydrate transport and metabolism			
PFLU4690	putative sugar kinase	-14.00	0.049
PFLU3274	putative permease transmembrane protein	2.12	0.024
PFLU1483	hypothetical protein	2.67	0.002
PFLU5810	putative cation ABC transporter membrane protein	3.97	0.001
PFLU4810	putative gluconate permease	2.37	0.025
Cell division and chromosome partitioning			
PFLU5545	putative peptidase	2.08	0.004
Cell envelope biogenesis, outer membrane			
PFLU3876	putative multidrug resistance protein A	2.21	0.000
PFLU2929	outer membrane component of multidrug efflux system	14.85	0.000
Cell motility and secretion / Signal transduction mechanisms			
PFLU3484	two-component system response regulator	-53.33	0.004
PFLU3379	methyl-accepting chemotaxis protein	6.51	0.000
Coenzyme metabolism			
PFLU4036	putative 4-aminobutyrate aminotransferase	2.73	0.006
Defense mechanisms			
PFLU4976	putative HlyD family secretion protein	-13.06	0.048
DNA replication, recombination, and repair			
PFLU3785	hypothetical protein	2.02	0.000
PFLU0148A	putative transposase	2.28	0.000

PFLU2138	putative DNA helicase	2.69	0.049
Energy production and conversion			
PFLU2399	putative oxidoreductase	4.98	0.002
PFLU1891	putative cytochrome C oxidase (monoheme and diheme subunits)	5.27	0.006
PFLU4680	putative NADP-dependent alcohol dehydrogenase	3.21	0.000
Inorganic ion transport and metabolism			
PFLU0201	putative ABC sulfur transporter substrate-binding protein	2.20	0.005
dgoT	D-galactonate transporter	2.48	0.004
phnD	phosphonate ABC transporter substrate-binding protein	2.49	0.005
PFLU1868	putative ABC transporter ATP-binding protein	5.81	0.000
PFLU2340	putative polyamine ABC transporter ATP-binding protein	8.56	0.004
ccmD	cytochrome C biogenesis, heme exporter protein	-∞	0.008
Lipid metabolism			
PFLU0450	putative acyl-CoA dehydrogenase	2.03	0.000
PFLU5785	hypothetical protein	2.04	0.002
PFLU2771	putative NAD(P)H dehydrogenase	14.06	0.040
Nucleotide transport and metabolism			
phnN	phosphorous compounds metabolism-related ATP-binding protein	-9.15	0.048
PFLU0493	putative membrane transporter-like permease	2.45	0.000
Posttranslational modification, protein turnover, chaperones			
PFLU5638	putative glutathione S-transferase	-8.39	0.042
PFLU4465	hypothetical protein	2.01	0.000
Secondary metabolites biosynthesis, transport and catabolism			
PFLU4766	acetyl-CoA synthetase	-7.77	0.000
PFLU0029	hypothetical protein	-3.27	0.005
PFLU4179	putative oxidoreductase	2.03	0.003
Signal transduction mechanisms			
PFLU5625	hypothetical protein	-4.94	0.004
PFLU2125	putative methyl-accepting chemotaxis protein	3.19	0.019
PFLU3016	putative two-component response regulator	2.53	0.000
Transcription			
PFLU4745	hypothetical protein	2.29	0.000
PFLU4114	putative AsnC family transcriptional regulator	2.44	0.001
PFLU3910	putative transcriptional regulator	2.64	0.000
PFLU0565	TetR family transcriptional regulator	3.45	0.000
PFLU0136	putative MarR family regulatory protein	4.51	0.009
PFLU2397	TetR family transcriptional regulator	18.56	0.001
PFLU3981	GntR family transcriptional regulator	2.22	0.024

Translation, ribosomal structure and biogenesis			
rpmC	50S ribosomal protein L29	2.28	0.000
frr	ribosome recycling factor	2.44	0.000
Function unknown/Hypothetical			
PFLU1813	hypothetical protein	-14.73	0.000
PFLU2066	hypothetical protein	-4.62	0.017
PFLU1032	hypothetical protein	2.10	0.000
PFLU3904	hypothetical protein	2.11	0.000
PFLU5919	putative lipoprotein	2.17	0.000
PFLU5221	hypothetical protein	2.20	0.009
PFLU0434	hypothetical protein	3.00	0.003
PFLU1460	hypothetical protein	3.89	0.049
pyrR	bifunctional pyrimidine regulatory protein PyrR uracil phosphoribosyltransferase	-13.14	0.008
PFLU1186	hypothetical protein	-4.86	0.016
PFLU3940	allantoate amidohydrolase	-3.22	0.004
PFLU5795	hypothetical protein	2.04	0.027
PFLU4974	hypothetical protein	2.20	0.048
PFLU3258	putative ABC transport membrane protein	2.21	0.044
PFLU0627	putative hydrolase	2.26	0.007
PFLU4957	hypothetical protein	2.29	0.018
phnP	carbon-phosphorus lyase complex accessory protein	2.45	0.000
PFLU3420	putative high-affinity branched-chain amino acid transport ATP-binding protein	3.40	0.001
PFLU4780	hypothetical protein	3.59	0.000
PFLU4049	putative dehalogenase	4.80	0.000
PFLU2129	hypothetical protein	6.98	0.024
PFLU2398	putative regulatory protein	9.27	0.000
PFLU2861	putative lipoprotein	-∞	0.006
PFLU4290	hypothetical protein	-∞	0.022
PFLU2450	hypothetical protein	-18.77	0.017
PFLU4555	putative cytochrome C oxidase subunit	-12.36	0.048
PFLU5320	hypothetical protein	-12.31	0.021
PFLU5939	hypothetical protein	-7.23	0.000
PFLU1413	putative transporter-like membrane protein	-5.31	0.006
PFLU0924	hypothetical protein	-3.37	0.003
PFLU5205	hypothetical protein	2.02	0.042
PFLU1768	hypothetical protein	2.04	0.001
PFLU4991	hypothetical protein	2.06	0.002
PFLU1700	hypothetical protein	2.15	0.000
PFLU3552	hypothetical protein	2.21	0.027
PFLU2983	putative lipoprotein	2.26	0.001

PFLU2820	hypothetical protein	2.32	0.000
PFLU1019	hypothetical protein	2.39	0.024
PFLU1395	hypothetical protein	2.45	0.000
PFLU3561	putative lipoprotein	2.52	0.000
PFLU3288	hypothetical protein	2.83	0.015
PFLU5134	hypothetical protein	2.87	0.000
PFLU2928	hypothetical protein	3.61	0.000
PFLU3765	hypothetical protein	6.46	0.010
PFLU2495	hypothetical protein	7.96	0.009
PFLUt29	tRNA-Leu	37.43	0.000
PFLU0566	hypothetical protein	49.17	0.000

Table S4 : Expression pattern of the putative efflux genes

Gene	Fold change	P value
PFLU2929	14.85	<0.00001
PFLU2930	14.59	0.03
PFLU2931	90.27	0.06
PFLU3875	-1.38	1.00
PFLU3876	2.21	<0.00001
PFLU3877	1.52	<0.00001