

Amari *et al.*,  
SUPPLEMENTARY MATERIAL

Table S1. Oligonucleotides

Oligonucleotide	Sequence
<b><u>RT-PCR</u></b>	
mreBf	CTGTCGATCGACCTGGG
mreBr	CAGCCATCGGCTCTTCG
dspIf	GACCTGACCCACAAGCTC
dspIr	GGCGAACATGTTCAGGTCG
<b><u>Directional Cloning</u></b>	
dspI_NheI_for	GCGCGCGCgctagcCATGATCCAGTGAGGGAC *
dspI_SacI_rev	GCGCGCGCgagctcGGGAAGGCTCAGCAGTTG*
dspI-PROM_XhoI-for	GCGCGCGCctcgagCAGGAAGTCCTCCGCCAG*
dspI-PROM_EcoRI-rev	GCGCGCGCgaattcGGTCGCCTCCGCTTCCG*
pMJT-MCS_for	GACCGCGAATGGTGAG
pMJT-MCS_rev	GAGCTGATACCGCTCG
attB_for	CGAGTGGTTTAAGGCAACGGTCTTGA
attB_rev	CGAGTTGTTCCACCAGGCCGA ACT
<b><u>PCR - <i>dspI</i> promoter determination</u></b>	
54610-a	GAGGTCTGTACGGAACGCTTC
54620-a	CAGCTGATCGACGGCGAATG
54620-b	GTGCGCTTCTATACCGAGACC
54630-a	CATCACTTCCCGGTGGAAGTC
54630-b	CAACGATTATCCGCTGGAGCG
54640-a	CCTACAAGGCTTCCTCCTTCG

\*restriction sites are indicated by nucleotides in lower case

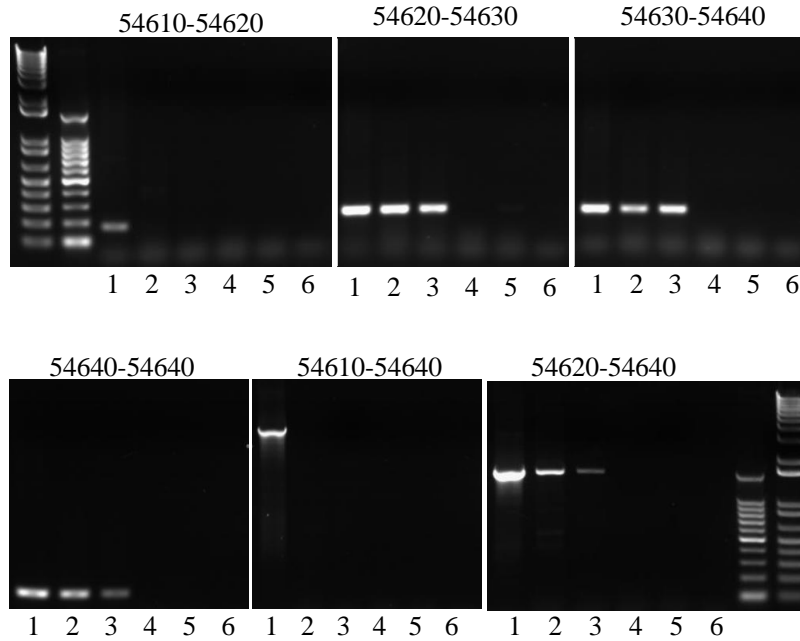
**A****B**

FIG. S1. The genes 54620 and 54630 are co-transcribed with *dspI* (54640). (A) The *dspI* operon in *P. aeruginosa*. (B) PCR amplification using oligonucleotide primers specific for genes in the *dspI* operon of PA14 genomic DNA (1), cDNA from planktonic or biofilm cells (2, 3), cDNA from planktonic or biofilm cells prepared with no reverse transcriptase (4, 5), or no DNA control (6). The promoter region for this operon was determined to lie upstream of the gene 54620.

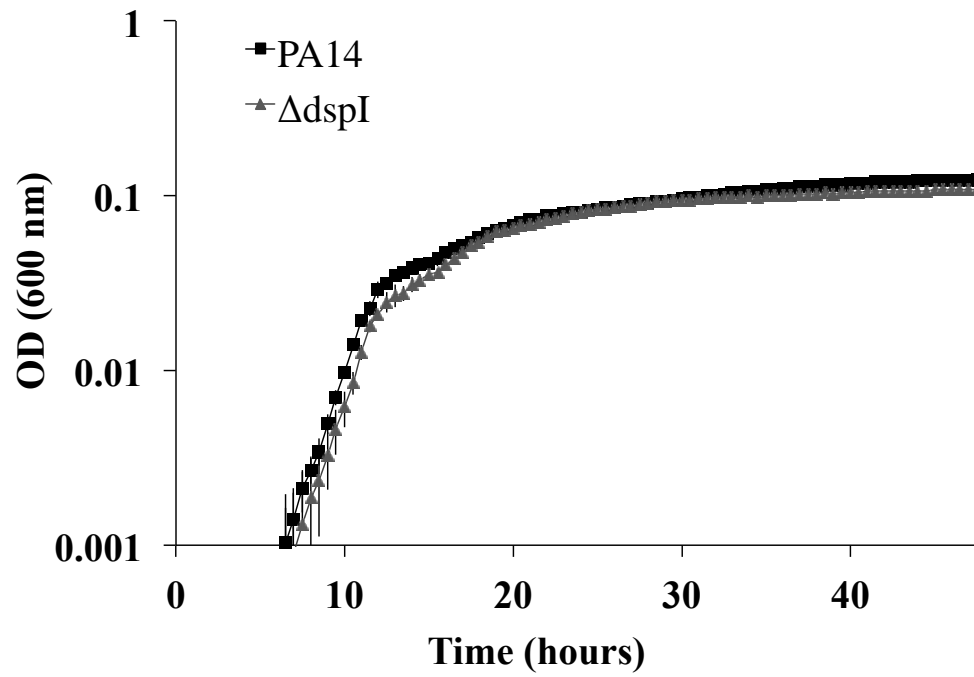


FIG. S2. Growth curves of *P. aeruginosa* PA14 and  $\Delta dspI$  in 5% LB medium. Mutation of *dspI* has no effect on bacterial growth rate. Each curve represents average of 20 replicates. Error bars indicate one standard deviation.

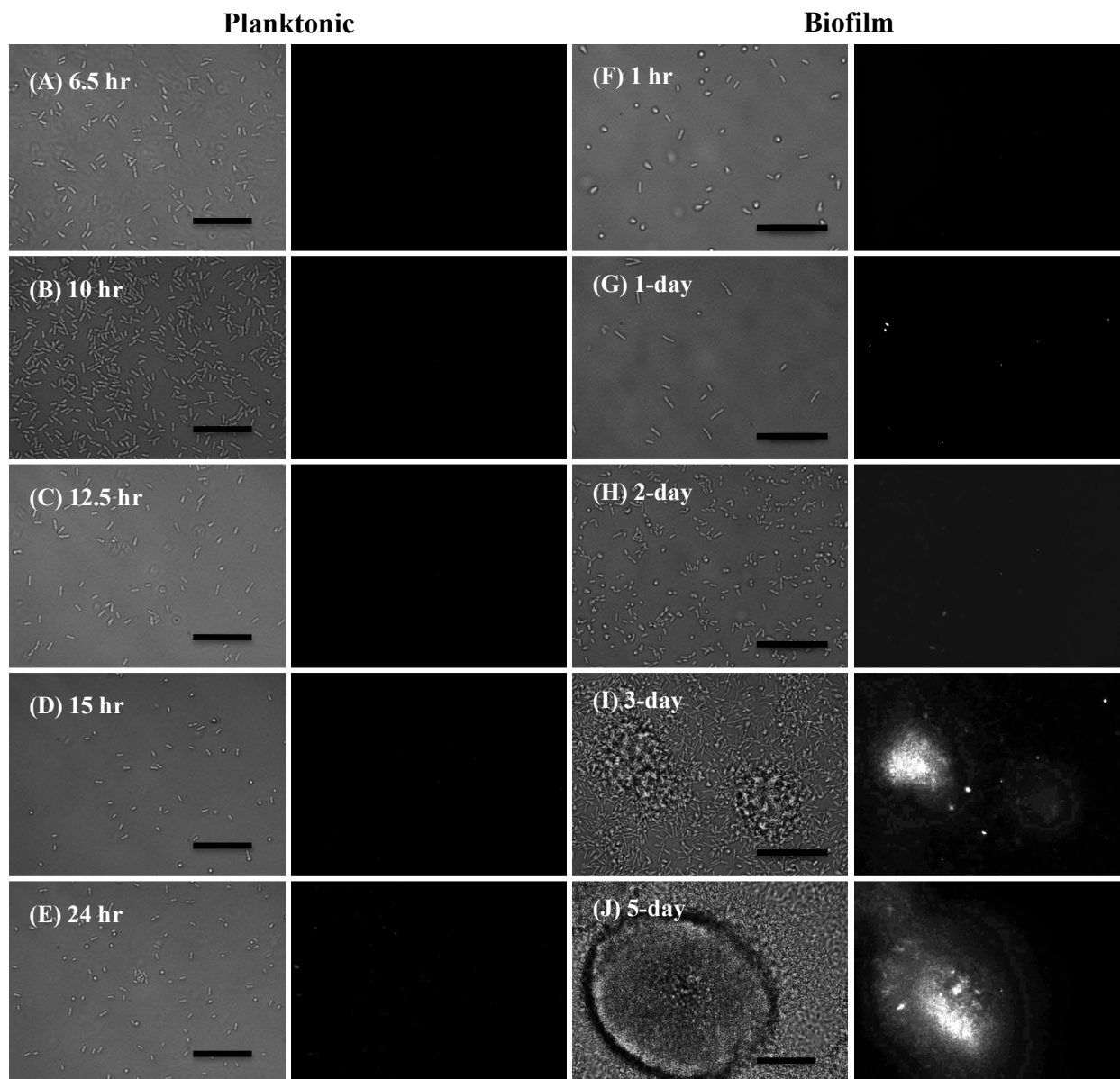


FIG S3. Microscopic analysis of *dspI* transcriptional reporter activity during planktonic and biofilm growth. *P. aeruginosa* PA14 harboring a promoterless *lacZ* construct was grown in batch or continuous culture in medium supplemented with MUG. The following planktonic conditions are shown: (A) 6.5 hr; (B) 10 hr; (C) 12.5 hr; (D) 15 hr; and (E) 24 hr. The following biofilm conditions are shown: (F) 1 hr; (G) 1-day; (H) 2-day; (I) 3-day; (J) 5-day. Bright field image (left panel) and fluorescent cells displaying  $\beta$ -galactosidase activity (right panel). Scale bar, 20  $\mu$ m.

## **SUPPLEMENTARY MATERIALS AND METHODS**

**Growth Curves.** Growth curves of *P. aeruginosa* PA14 and  $\Delta dspI$  were performed on cultures grown in 96-well microtiter plates at 23°C. Overnight cultures were grown in LB, OD 600 adjusted, and diluted 1:100 into 5% LB. Absorbance readings (OD 600) were taken every 30 minutes for 48 hours. All growth curves were carried out in triplicate, with 20 replicates per strain.