

Table S1. Bacterial strains, plasmids, and primers used in this study.

Bacterial strain or plasmid	Description	Reference or source
<u><i>P. aeruginosa</i></u>		
PAO1	Wild type strain	(1)
PAO1 $\Delta lasR$	PAO1 containing an unmarked, in-frame <i>lasR</i> deletion	(2)
PAO1 $\Delta lasI$	PAO1 containing an unmarked, in-frame <i>lasI</i> deletion	(3)
PAO1 Δnuh	PAO1 containing an unmarked, in-frame <i>nuh</i> deletion	This work
PAO1 $\Delta PA0148$	PAO1 containing an unmarked, in-frame <i>nuh</i> deletion	This work
<u>PAO1 variants</u>		
A	Isolated after daily transfer in 0.75% adenosine / 0.25% casein for 25 days	(4)
B	Isolated after daily transfer in 0.75% adenosine / 0.25% casein for 25 days	(4)
C	Isolated after daily transfer in 0.9% adenosine / 0.1% casein for 14 days	This work
D	Isolated after daily transfer in 0.9% adenosine / 0.1% casein for 14 days	This work
E	Isolated after daily transfer in 0.9% adenosine / 0.1% casein for 14 days	This work
<i>E. coli</i> DH5 α	F ⁻ $\Phi 80 lacZ \Delta M15 \Delta(lacZYA-argF)$ U169 <i>recA1 endA1 hsdR17</i> (r _k ⁻ , m _k ⁺) <i>phoAsupE44 thi-1 gyrA96 relA1 λ</i> ⁻	Invitrogen
<u>Plasmids</u>		
pJN105	<i>araC</i> -P _{Bad} cassette cloned in pBBR1MCS-5, Gm ^r	(5)
pJN105.nuh	Arabinose inducible <i>nuh</i> in pJN105, Gm ^r	This work
pPROBE-GT	Broad-host-range pVS1/p15a GFP reporter, Gm ^r	(6)
pBBR1MCS-5	Medium copy, BHR plasmid vector (pBBR), Gm ^r	(7)
Pnuh-wt	pPROBE-GT with the -91 through 15 of the 5' region of <i>nuh</i> from PAO1 inserted with HindIII and BamHI, Gm ^r	This work
Pnuh-var	pPROBE-GT with the -91 through 15 of the 5' region of <i>nuh</i> from variant A inserted with HindIII and BamHI, Gm ^r	This work
pJT3	pBBR1MCS with PA0148 inserted with HindIII and SacI, Gm ^r	This work
pJT4	pBBR1MCS with <i>nuh</i> inserted with HindIII and SacI, Gm ^r	This work
pJT5	pBBR1MCS with PA0144 inserted with HindIII and SacI, Gm ^r	This work
pJT7wt	pBBR1MCS with PA0142 through PA0148 assembled from PAO1, Gm ^r	This work
pJT7var	pBBR1MCS with PA0142 through PA0148 assembled from Variant A, Gm ^r	This work
pJT10	pBBR1MCS with PA0143 and PA0148 assembled from PAO1, Gm ^r	This work

Primers

Pnuh.BamHI R	5' – AAAAAGGATCCCAAAGCGATTGCATGGGCG – 3'
Pnuh.HindIII F	5' – TTTTAAAGCTTGTTGATGCGGGTTCCGGTA G – 3'
nuhF300	5' – CTCGGCGCTGCTGCTGTGCG – 3'
nuhR300	5' – GGACAGCGGCACCGCCTTCG – 3'
pJT3 F.PA0148.HindIII	5' – TTCAAGCTTCTACTCCATGCCGTTCTTCG – 3'
pJT3 R.PA0148.SacI	5' – TTCGAGCTCCAGAAGCGCAGGAACAGTTC – 3'
pJT4 F.nuh.HindIII	5' – TTCAAGCTTCTCGGCGCTGCTGCTGTGCG – 3'
pJT4 R.nuh.SacI	5' – TTCGAGCTCGGACAGCGGCACCGCCTTCG – 3'
pJT5 F.PA0144.HindIII	5' – TTCAAGCTTTATATGCCTACCTCAGCGATG – 3'
pJT5 F.PA0144.SacI	5' – TTCGAGCTCCTACGTCCAGTACGACATCAA – 3'
pJT7.F	5' – GGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGACGCCTCGAACATGATCCTC – 3'
pJT7.R	5' – CACACAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCATCAATACCGAGAAGCCGG – 3'
pJT7_V.F	5' – GCAAGGCGTTTCGTCGTCGCCGGCTTCTCGGTATTGATGCGCGCTTGGCGTAATCATGG – 3'
pJT7_V.R	5' – ACAGGGCCTGGCGCGCCTCGAGGATCATGTTTCGAGGCGTCCTGGCGTTACCCAACTTAA – 3'
pJT10_PA0148 F	5' – GCATAGCCGACCTCGAAGGCGGTGCCGCTGTCCCTACTCCATGCCGTTCTTCGC – 3'
pJT10_nuh R	5' – GTCCATGTGCGGTTTCGGCGAAGAACGGCATGGAGTAGGGACAGCGGCACCGCCTTCG – 3'
pJT11.D1	5' – TGTAAGCAAGCTTCTGCAGGTCGACTCTAGAGGATCGCCTGCTGGTGGAACAGATC – 3'
pJT11.D2	5' – GCTTTTCAGCGCAGGCTCCAGAAACCCAGGCGATGCCGAACACGTCCGGCTC – 3'
pJT11.D3	5' – GCCGCCTACGAGCCGGACGTGTTTCGGCATCGCCTGGGTTTCTGGAGC – 3'
pJT11.D4	5' – CGTGGAAATTAATTAAGGTACCGAATTCGAGCTCGAGGCCGCTGCTGGTCTTCATG – 3'
pJT11_V.F	5' – GACCCGCGGATTTTCTGCTCAATCGCATCCACGGCTCGAGCTCGAATTCGGTACC – 3'
pJT11_V.R	5' – GCTACGGGTGATATCGATCTTCAGCTTCTCTTCGACGGGGATCCTCTAGAGTCGAC – 3'

REFERENCES

1. **Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warren P, Hickey MJ, Brinkman FSL, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrook-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim R, Smith K, Spencer D, Wong GK-S, Wu Z, Paulsen IT, Reizer J, Saier MH, Hancock REW, Lory S, Olson M V.** 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406**:959–964.
2. **Wang M, Schaefer AL, Dandekar AA, Greenberg EP.** 2015. Quorum sensing and policing of *Pseudomonas aeruginosa* social cheaters. *Proc Natl Acad Sci U S A* **112**:2187–2191.
3. **Scholz RL, Greenberg EP.** 2017. Positive autoregulation of an acyl-homoserine lactone quorum-sensing circuit synchronizes the population response. *mBio* **8**:e01079-17
4. **Dandekar AA, Chugani S, Greenberg EP.** 2012. Bacterial quorum sensing and metabolic incentives to cooperate. *Science* **338**:264 LP-266.
5. **Newman JR, Fuqua C.** 1999. Broad-host-range expression vectors that carry the l-arabinose-inducible *Escherichia coli* *araBAD* promoter and the *araC* regulator. *Gene* **227**:197–203.
6. **Miller WG, Leveau JHJ, Lindow SE.** 2000. Improved *gfp* and *inaZ* Broad-Host-Range Promoter-Probe Vectors. *Mol Plant-Microbe Interact* **13**:1243–1250.
7. **Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, Peterson KM.** 1995. Four new derivatives of the broad host range cloning vector PBBR1MCS, carrying different antibiotic resistance cassettes. *Gene* **166**:175–176.