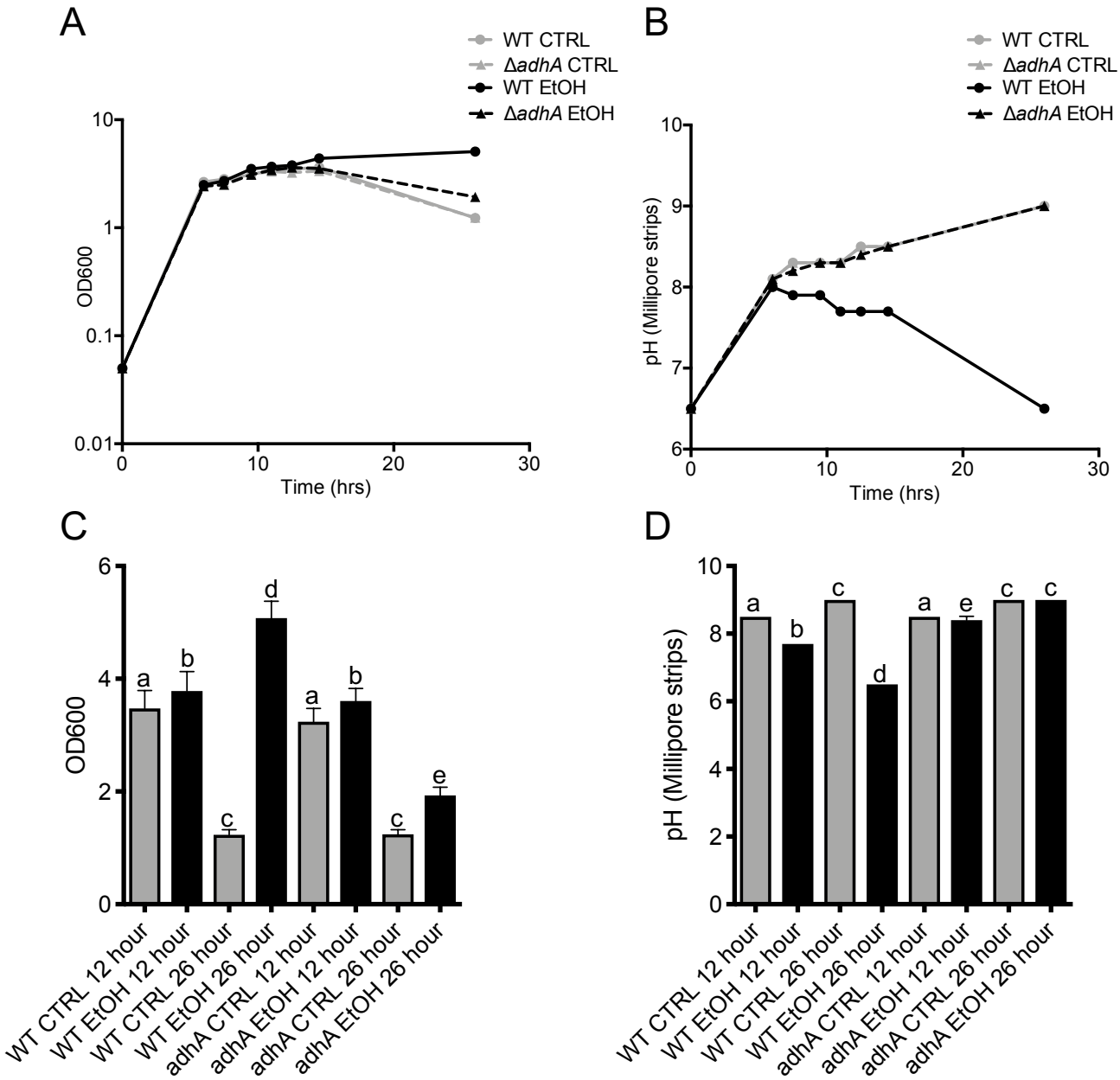

Figure S1. Culture pH in LB with 1% ethanol begins to deviate from LB alone near the transition from exponential to stationary phase around an OD_{600} of 3.0. Cultures were grown in LB alone or LB amended with 1% ethanol at 37°C, with an initial inoculation of 0.05 OD_{600}. Optical density (A) and pH measurements (B) were taken beginning at 6 hours post-inoculation. Each point is the mean of 4 independent cultures and data are representative of at least 2 independent experiments. Panels C and D represent the mean and standard deviation of the 4 individual cultures in A and B at 12 hours and 26 hours post-inoculation. Letters above each bar represent statistical groupings for OD_{600} (C) and pH (D).

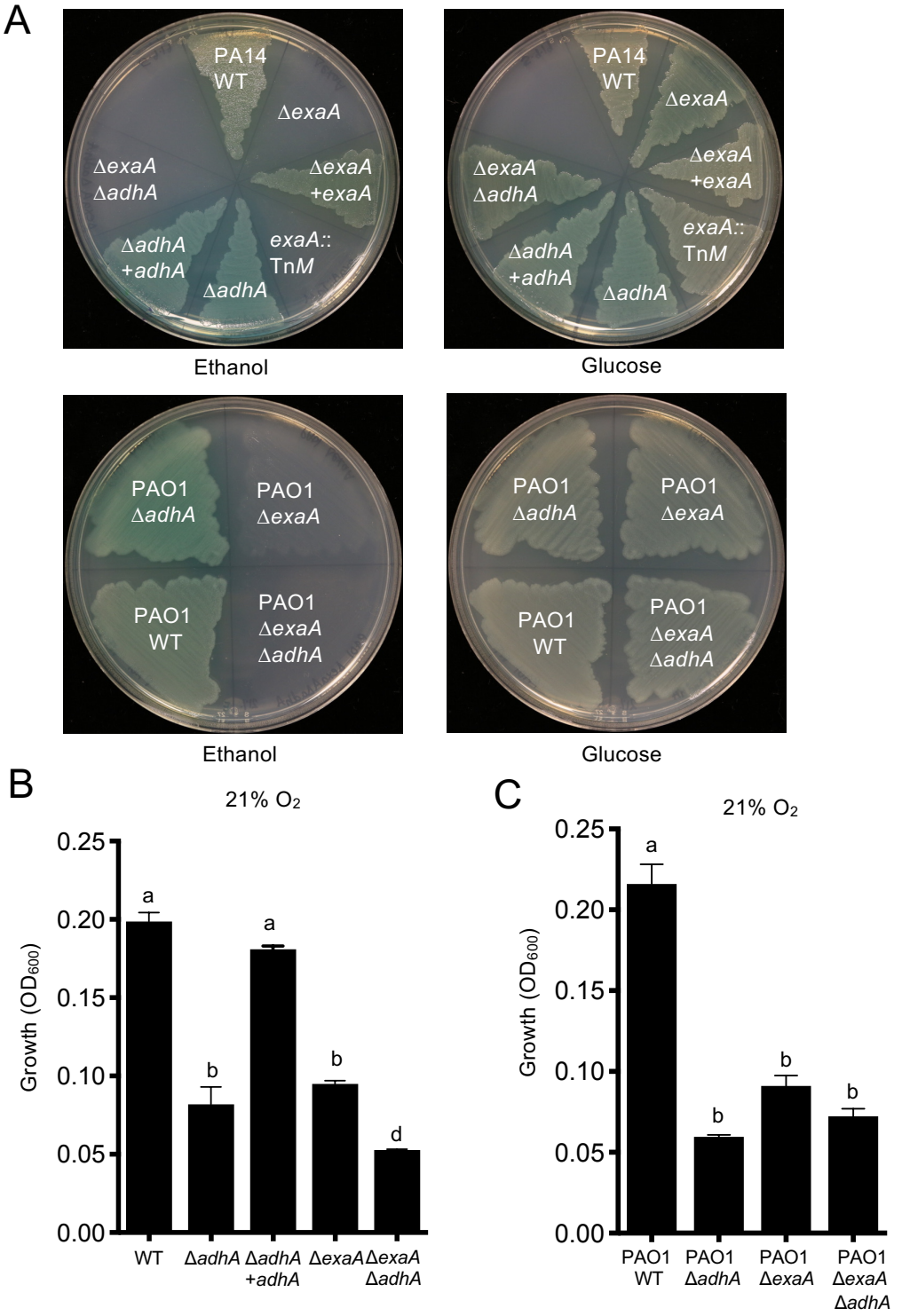

Figure S2. Growth of *P. aeruginosa* strains on ethanol as a sole carbon source. **A**. *P. aeruginosa* PA14 (top) and PAO1 (bottom) wild types and their *exaA* or *adhA* derivative and mutants after complementation. In each strain background, an Δ*exaA*Δ*adhA* strain is also shown. All strains were grown on 1.5% agar M63 with either ethanol or glucose, as indicated, as the sole carbon source at 37 C for 24 h. Data are representative of at least three independent experiments. **B and C**. Growth of PA14 and PAO1 strains listed in M63 liquid with ethanol as the sole carbon source in 21% oxygen. Statistics based on one-way ANOVA and Tukey's multiple comparison; between group comparisons were significant with a $P<0.001$ in panel B and $P<0.0001$ in panel C.

Table S1. Strains used in this study.

Strain	Lab Strain	Strain description	Source
P. aeruginosa			
PA14 WT	DH123	Laboratory reference strain	(1)
PA14 *exaA*::Tn*M*	DH2130	Transposon insertion mutant in *exaA*	(2)
PA14 Δ*exaA*	DH3470	In-frame deletion of *exaA*	This study
PA14 Δ*exaA*+*exaA*	DH2677	Complementation of *exaA* at the native locus	This study
PA14 Δ*adhA*	DH2255	In-frame deletion of *adhA*	This study
PA14 Δ*adhA*Δ*exaA*	DH2257	In-frame deletion of *adhA* and *exaA*	This study
PA14 Δ*adhA*+*adhA*	DH3463	Complementation of *adhA* at the native locus	This study
PA14 *pqqB*::Tn*M*	DH2131	Transposon insertion mutant in *pqqB*	(2)
PA14 *acsA*::Tn*M*	DH2168	Transposon insertion mutant in *acsA*	(2)
PA14 Δ*anr*	DH2855	In-frame deletion of *anr*	This study
PA14 Δ*anr* +*anr*	DH3478	PA14 Δ*anr* complemented with *anr* at the native locus	This study
PA14 Δ*lasR*	DH164	in-frame deletion of *lasR*	(3)
PA14 Δ*lasR* +*lasR*	DH3398	PA14 Δ*lasR* complemented with *lasR* at the native locus	(4)
PA14 Δ*lasR* Δ*anr*	DH2401	*anr* deleted in DH164	(5)
PA14 Δ*lasR* Δ*adhA*	DH3464	In-frame deletion of *lasR and adhA* in DH164	This study
Δ*adhA* + EV	DH3475	DH2255 carrying DH1682	This study
Δ*adhA* + *adhA* OE	DH3476	DH2255 carrying DH3483 *adhA* expression vector	This study
Δ*anr* + EV	DH3477	DH2855 carrying DH1682	This study
Δ*anr* + *adhA* OE	DH3474	DH2855 carrying DH3483 *adhA* expression vector	This study
Δ*anr*+*anrD149A*	DH3480	PA14 with wild-type *anr* replaced with *anrD149A* allele	This study
PAO1 WT	DH1856	Laboratory reference strain	(6)
PAO1 Δ*adhA*	DH2252	In-frame deletion of *adhA* in DH1856	This study

E. coli			
S17 ƛ pir	DH71		
Plasmids			
pMQ30	DH962	allelic replacement vector, GmR	(7)
pMQ70 EV	DH1682	pMQ70 expression vector	(7)
p*adhA* OE	DH3483	p*adhA* expression vector in pMQ70	This study
padhA KO	DH2159	*adhA* in-frame deletion construct in pMQ30	This study
padhA complement	DH2162	*adhA* complementation construct in pMQ30	This study
anrD149A allele	DH3482	*anrD149A* allele replacement vector in pMQ30	This study
anr complement	DH3481	*Anr* complementation construct in pMQ30	This study

References

1. Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. 1995. Common virulence factors for bacterial pathogenicity in plants and animals. Science 268:1899-902.
2. Feinbaum RL, Urbach JM, Liberati NT, Djonovic S, Adonizio A, Carvunis AR, Ausubel FM. 2012. Genome-wide identification of *Pseudomonas aeruginosa* virulence-related genes using a *Caenorhabditis elegans* infection model. PLoS Pathog 8:e1002813.
3. Hogan DA, Vik A, Kolter R. 2004. A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol Microbiol 54:1212-23.
4. Harty CE, Martins D, Doing G, Mould DL, Clay ME, Occhipinti P, Nguyen D, Hogan DA. 2019. Ethanol stimulates trehalose production through a SpoT-DksA-AlgU-dependent pathway in *Pseudomonas aeruginosa*. J Bacteriol 201.
5. Hammond JH, Dolben EF, Smith TJ, Bhuju S, Hogan DA. 2015. Links between Anr and quorum sensing in *Pseudomonas aeruginosa* biofilms. J Bacteriol 197:2810-20.
6. Vallet-Gely I, Sharp JS, Dove SL. 2007. Local and global regulators linking anaerobiosis to *cupA* fimbrial gene expression in *Pseudomonas aeruginosa*. J Bacteriol 189:8667-76.
7. Shanks RM, Caiazza NC, Hinsa SM, Toutain CM, O'Toole GA. 2006. *Saccharomyces cerevisiae*-based molecular tool kit for manipulation of genes from gram-negative bacteria. Appl Environ Microbiol 72:5027-36.

Table S2. Primers used in this study.

Tn mutant confirmation primers		
Gene	**Primer**	**Sequence**
exaA	exaA Tn conf_FWD	ACAACGTGTTCAAGCTGAC
	exaA Tn conf_REV	ACACCTTGTCGCCATAGA
Strain construction and sequencing		
Gene/ Feature	**Primer**	**Sequence**
adhA	DH_SDW1	ATCTGCACGATGGAGAGCAGCGA
	DH_SDW2	TTGCAGATGGACCCGGAGAT
	DH_SDW3	AGCGGCCAGACTGGTTGT
	DH_SDW4	TACAAGGGGCTCAAGCAGAC
	Out_1	TGAACATCGACTTCTCGAACCAGC
	Out_2	ATGACGGGATTTGTCTCAGAGGGA
	Rec_FW	ACACTAGTCATGGTTCGAGCCTTACC
	Rec_RV	TTAAGCTTAAGTGGCTGGAGGATTTC
exaA		
	DH_SDW5	GCTTCTGCGTTCTGATTTAATCTGTATC AGGCTGATAGATCATGGCGAAGCTCTC CTGT
	DH_SDW6	GGCAGCTTGAATACCCAGAAGGAACGT TTGGAGTGAGGGTCGAGAAA
	DH_SDW7	TTTCTCGACCCTCACTCCAAACGTTCCT TCTGGGTATTCAAGCTGCC
	DH_SDW8	GAGCGGATAACAATTTCACACAGGAAA CAGCTATGTGCTCAAGCAGAAGTTGCA GGTGA
	exaA_sequencing_1	CGCGCGCAGTTCTGGTTGTA
	exaA_sequencing_2	TTTCCAGGTTGGCCAGGTCGAG
	exaA_KO_Chk_UP_FW	GAACGGGGGAACTCGGCGATC
	exaA_KO_Chk_Dwn_RV	CGTGGGAAGAGCAGGGCGG

anr	^Anr_pmQ30_1	TGCGTTCTGATTTAATCTGTATCAGGCTGATGTTCATGAACTGGGTCATGAAGGGTTGGC
	^Anr_pmQ30_2	GTGCCTTTAACCTAGCAAGGACCCCTCAAGCGCCTGCGAACCGCCAAC
	^Anr_pmQ30_3	GTTGGCGGTTCGCAGGCGCTTGAGGGGTCCTTGCTAGGTTAAAGGCAC
	^Anr_pmQ30_4	TGAGCGGATAACAATTTCACACAGGAAACAGCTATGTCTGCCACTTCGAACTGGCCTTCG
	Anr_Chk_FW1	CGCGCGGTGATCCAGCAACTG
	Anr_Chk_RV1	GTAGGGGAAGAGCGAGGACAGG
pMQ30	pMQ30_seq_mcs_FW	CCTCTTCGCTATTACGCCAGCTGG
	pMQ30_seq_mcs_RV	GCTCACTCATTAGGCACCCCAGG