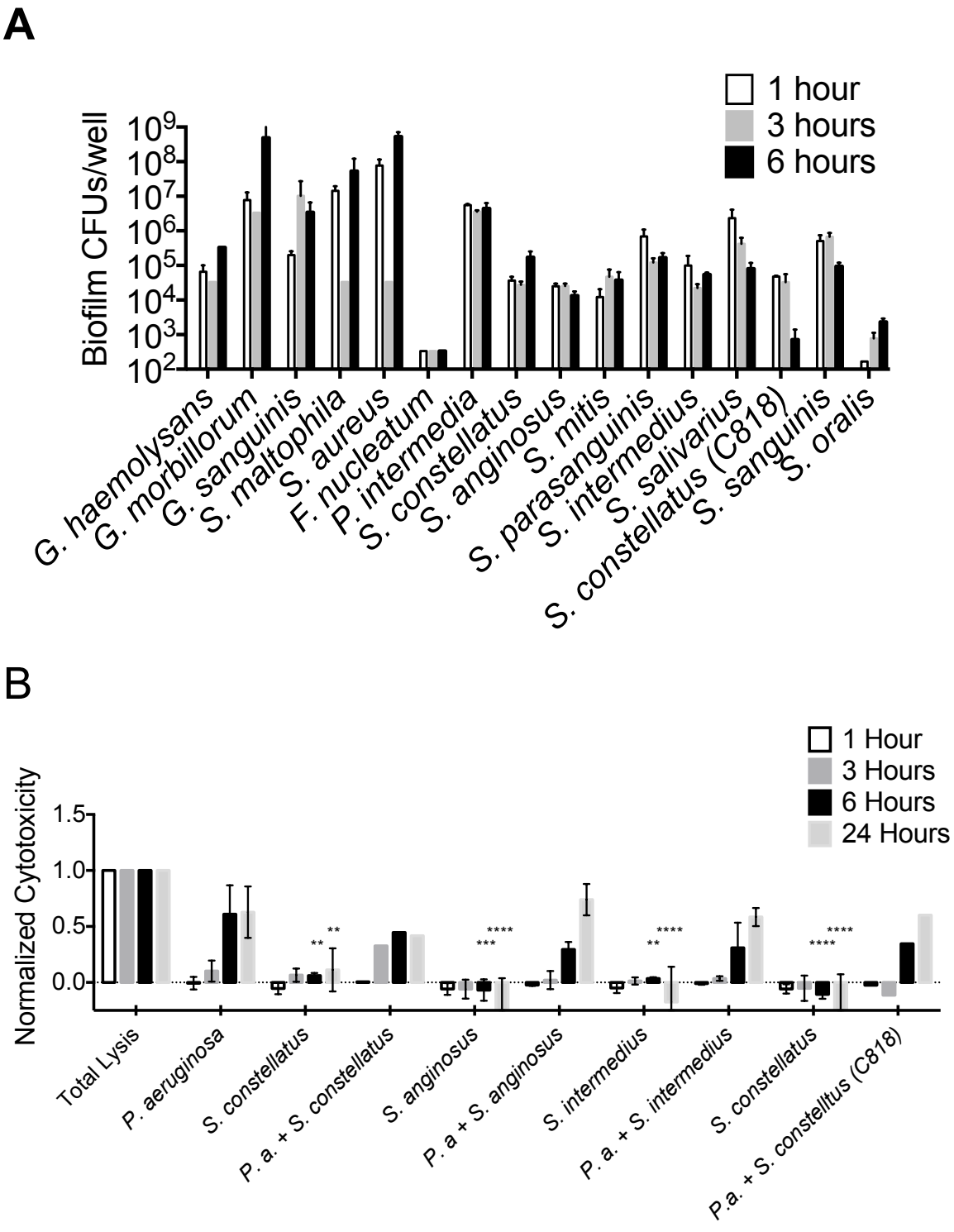
SUPPLEMENTAL MATERIALS

SUPPLEMENTAL FIGURES

Figure S1



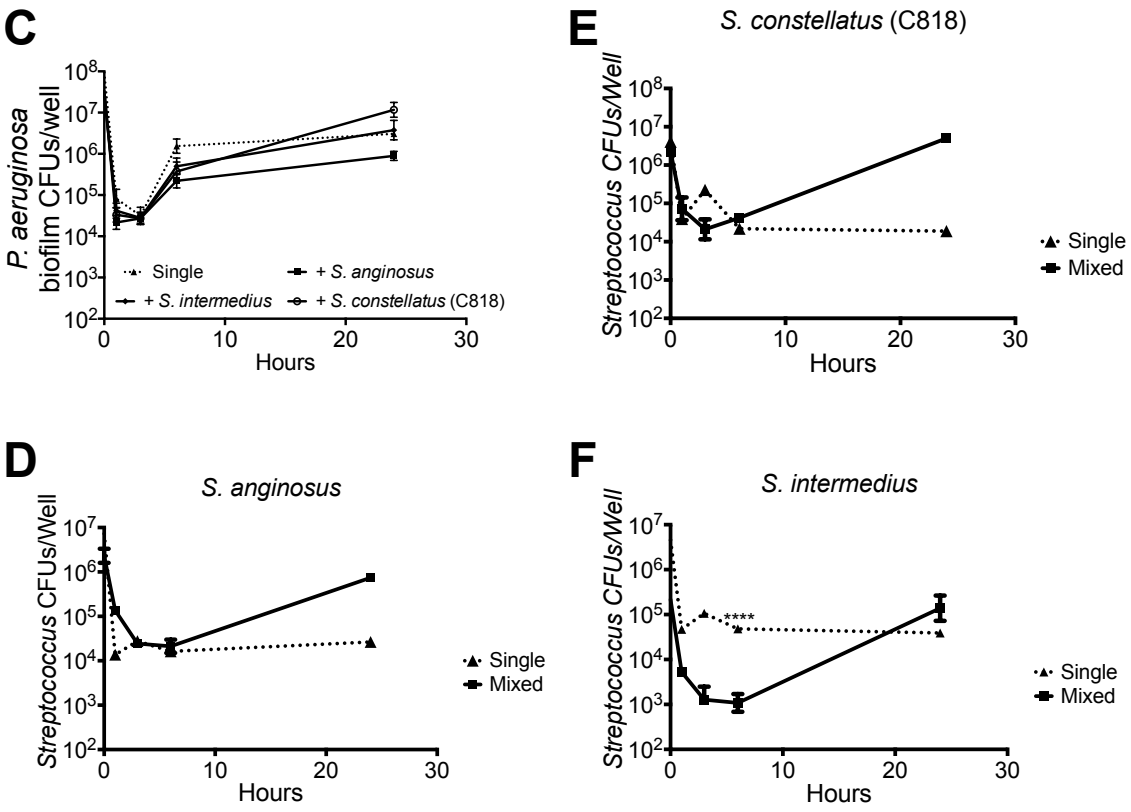


Figure S1. Growth kinetics and cytotoxicity of CF-relevant bacteria grown as biofilms on CFBE cells. A) Viability of bacteria grown as single species biofilms on CFBE cells at one (white columns), three (gray columns) and six hours (black columns) post inoculation. Columns are the mean of at least three biological replicates, error bars indicate the standard deviation. B) Cytotoxicity of *P. aeruginosa*, *S. anginosus, S. intermedius*, *S. constellatus*, as indicated grown as single species biofilms or mixed biofilms with *P. aeruginosa*, as indicated on CFBE cells at one (white columns), three (dark gray columns), six hours (black columns) and twenty-four hours (light gray columns) post inoculation. Cytotoxicity was measured as percentage of LDH release compared to total lysis control of uninfected CF airway cells treated with Triton X-100 detergent to completely lyse cells and release all intracellular LDH using the Cyto Tox 96 Non-radioactive Cytotoxicity Kit (Promega) according to the manufacture's instructions. Columns are the mean of at least two biological replicates, error bars indicate the standard deviation. **, ***, **** indicates P< 0.01, <0.001, <0.0001, respectively compared to *P. aeruginosa* alone at each time point, determined by two-way ANOVA followed by Dunnett's test for multiple comparisons. C) Viability of *P. aeruginosa* grown as single species biofilms on CFBE cells (dashed line); Viability of *P. aeruginosa* grown as mixed biofilms on CFBE cells with *S. anginosus, S. intermedius*, *S. constellatus*, as indicated (solid line). Symbols, mean of three biological replicates; error bars, SD. D-F) Viability of indicated *Streptococcus* species grown as single biofilms on CFBE cells (dashed line); Viability of indicated *Streptococcus* species grown as mixed biofilms on CFBE cells with *P. aeruginosa* (solid line). Symbols, mean of three biological replicates; error bars, SD. The difference between *P. aeruginosa* and *S. intermedius* at t = 6 hrs is significant; no other differences are significant (unpaired T-test, P < 0.05). For the

studies in this figure, *Gemella haemolysans, Gemella morbillorum, Gemella sanguinis, Streptococcus anginosus, Streptococcus constellatus, Streptococcus constellatus, Streptococcus intermedius, Streptococcus mitis, Streptococcus oralis, Streptococcus parasanguinis, Streptococcus salivarius, Streptococcus sanguinis* were grown on blood agar and Todd Hewitt broth supplemented with 0.5% yeast extract (THY) and 20 µL/mL oxyrase (Oxyrase, Inc.) statically at 37°C in a 5% CO_2 atmosphere. *Fusobacterium nucleatum* and *Prevotella intermedia* were grown on blood agar, incubated anaerobically in GasPak jars and in Todd Hewitt broth supplemented with 0.5% yeast extract (THY) and 20 µL/mL oxyrase (Oxyrase, Inc.) statically at 37°C in a 5% CO_2 atmosphere. *Staphylococcus aureus, Staphylococcus epidermidis* were grown on blood agar and Todd Hewitt broth supplemented with 0.5% yeast extract (THY) without the addition of oxyrase (Oxyrase, Inc.) shaking at 37°C. *Stenotrophomonas maltophilia* was grown on LB agar or liquid shaking at 37°C.

Figure S2

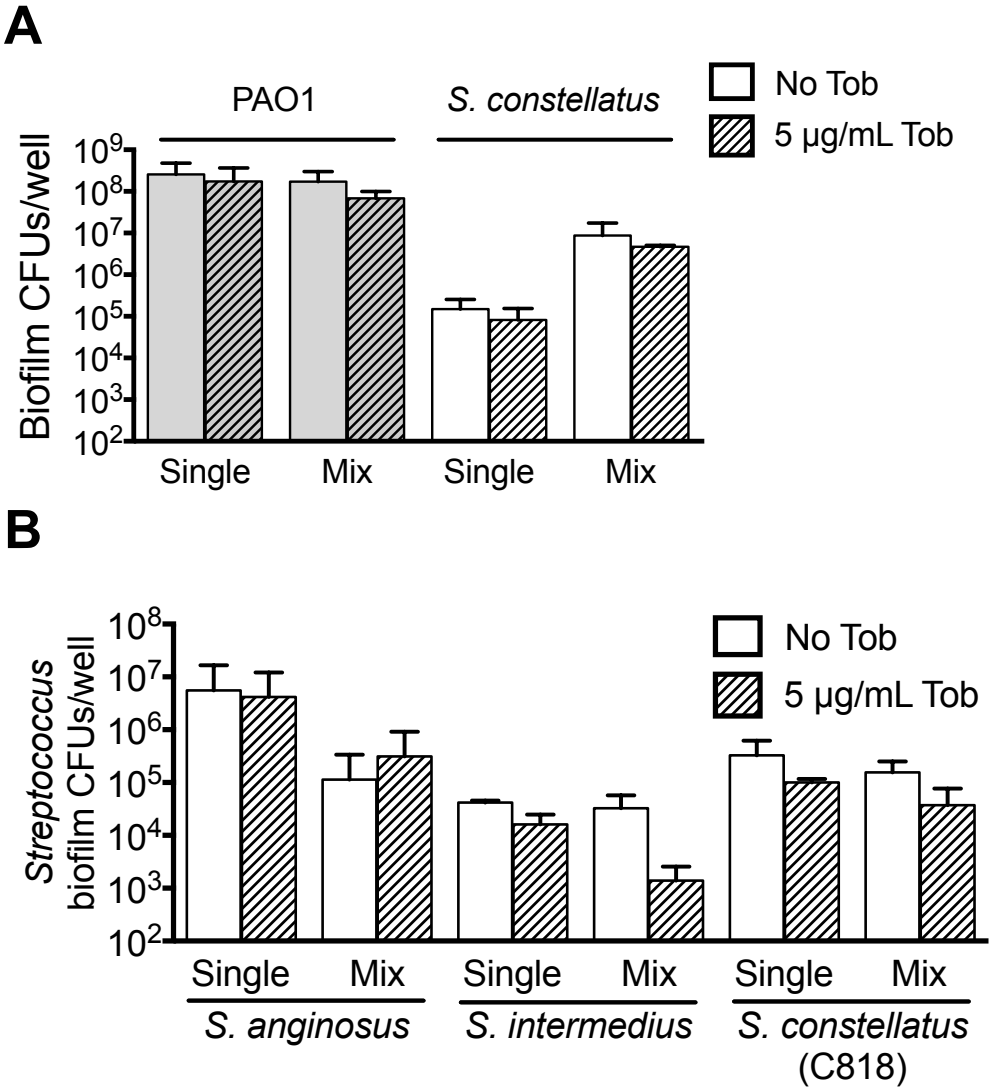


Figure S2. Strain specificity of tobramycin enhancement of *S. constellatus* growing in a biofilms. A) Viability of bacteria grown as biofilms on CF airway cells from assays of single or mixed biofilms of *P. aeruginosa* strain PAO1 (gray columns) and *S. constellatus* 7155 (white columns) treated with no tobramycin (open columns) or with 5 µg/mL of tobramycin (hatched columns). There were no significant changes in CFUs in single versus mixed communities ($P > 0.05$). B) Viability (CFUs/well) of *Streptococcus* grown as biofilms on CFBE cells from assays of single or mixed biofilms of *S. anginosus, S. intermedius* or *S. constellatus* strain C818 with *P. aeruginosa* PA14, as indicated treated with MEM (open columns) or with 5 µg/mL of tobramycin (hatched columns). Columns and error bars indicate the mean and S.D. of at least two biological replicates. There were no significant changes in CFUs in single versus mixed communities, in the presence or absence of tobramycin ($P > 0.05$).

Supplemental Table S1. Bacterial strains used in this study.

***P. aeruginosa* strains**	**SMC strain number or location in non-redundant library**	**Relevant genotype**	**Source**
PA14	232	Wild-type PA14	(1)
PA14 GFP	241	PA14, pSMC21-GFP, Amp^R constitutively active GFP	
algR	12_1 D1	*algR::Tn,* $Gent^R$	(2)
algU	1255	*algU::Tn5phoA*, Kn^R	D. Hogan
betB	13_3 C10	*betB::Tn,* $Gent^R$	(2)
betI	10_3 G8	*betI::Tn,* $Gent^R$	(2)
bifA	3351	*ΔbifA*	(3)
bkdR	11_4 E11	*bkdR::Tn,* $Gent^R$	(2)
creB	09_2 C6	*creB::Tn,* $Gent^R$	(2)
fptA	09_4 D5	*fptA::Tn,* $Gent^R$	(2)
fpvA	10_4 H1	*fpvA::Tn, G*ent^R	(2)
gntR	04_4 G10	*gntR::Tn,* $Gent^R$	(2)
hcnA	10_2 F12	*hcnA::Tn,* $Gent^R$	(2)
hcnB	09_3 A10	*hcnB::Tn,* $Gent^R$	(2)
hcnC	15_1 A4	*hcnC::Tn,* $Gent^R$	(2)
lasB	05_2 F6	*lasB::Tn,* $Gent^R$	(2)
lasR	5021	*ΔlasR*	(4)
lasRrhlR	5023	*ΔlasR, rhlR::tet*^{R,} Tet^R	(5)
metR	12_1 F10	*metR::Tn,* $Gent^R$	(2)
mgtE	4349	*ΔmgtE,* pMQ70, Amp^R	(6)
np20	10_3 C3	*np20::Tn,* $Gent^R$	(2)
nvdB	1759	Δ*nvdB*	(7)
pelA	2893	*pelA*	(8)
phnAB	5019	Δ*phnAB*	D. Hogan
phoB	4162	*phoB*, $Gent^R$	This study
phz 1/2	4189	Δ*phzA1-G1, ΔphzA2-G2*, pYFP	(9)
phz1/pvdApchE	4191	Δ*phzA1-G1, ΔphzA2-G2*, *ΔpvdA,* Δ*pchE,* pYFP	(9)
phzA1-G1 A2-G2	5020	Δ*phzA1-G1, ΔphzA2-G2*	(10)
pqsA	5013	Δ*pqsA*	D. Hogan
pqsE	5016	Δ*pqsE*	(10)
pqsH	5017	Δ*pqsH*	(5)
pqsR	5018	Δ*pqsR*	(5)
pvdA/pchE	4190	Δ*pvdA, ΔpchE* pYFP	(9)
pvdS	06_2 G5	*pvdS::Tn,* $Gent^R$	(2)

rhlA	1353	*rhlA::Gm*, Gent^R	(11)
rhlB	2758	*rhlB::kn*, Kn^R	(12)
rhlC	2759	Δ*rhlC*	(12)
rhlR	5022	*rhlR::tet*, Tet^R	(13)
roeA	3812	Δ*roeA*	(14)
rpoS	05_3 A2	*rpoS::Tn,* Gent^R	(2)
sadB	705	*sadB::Tn5B21*, Tet^R	(15)
sadC	3335	Δ*sadC*	(16)
sadCroeA	3809	Δ*sadC,* Δ*roeA*	(14)
spuF	11_3 F1	*spuF::Tn,* gent^R	(2)
Other strains	**SMC strain number**	**Strain**	**Source**
Fusobacterium nucleatum	5370	ATCC# 42956	ATCC
Gemella haemolysans	5359	ATCC# 10379	ATCC
Gemella morbillorum	5358	ATCC# 27824	ATCC
Gemella sanguinis	5386	ATCC# 700632	ATCC
Prevotella intermedia	4804	ATCC# 25611	ATCC
Staphylococcus aureus	643	Newman	A. Cheung
Staphylococcus epidermidis	380	ATCC# R97-03	ATCC
Staphylococcus epidermidis	381	ATCC# R94-10	ATCC
Stenotrophomonas maltophilia	4920	K279a	R. Ryan
Streptococcus anginosus	5342	C238	M.G. Surette
Streptococcus constellatus	7155	Clinical isolate designated strain 7155	N. Jacobs
Streptococcus constellatus	5343	C818	M.G. Surette
Streptococcus intermedius	7156	Clinical isolate	N. Jacobs
Streptococcus mitis	7157	Clinical isolate	N. Jacobs
Streptococcus oralis	5355	ATCC# 35037	ATCC
Streptococcus parasanguinis	5357	ATCC# 15912	ATCC
Streptococcus salivarius	7158	Clinical isolate	N. Jacobs
Streptococcus sanguinis	5356	ATCC# 10556	ATCC

Table S2. Candidate genes and phenotypes on plastic.

	S. constellatus 7155 Viability[a]	
P. aeruginosa mutant	0 µg/mL Tob	5 µg/mL Tob
algR	WT	WT
algU	WT	WT
betB	WT	WT
betI	WT	WT
bifA	Low	High
bkdR	WT	WT
creB	WT	WT
fptA	WT	WT
fpvA	WT	WT
gntR	WT	WT
hcnA	WT	WT
hcnB	WT	WT
hcnC	WT	WT
lasB	WT	WT
lasR	High	WT
lasR/rhlR	WT	WT
metR	WT	WT
mgtE	WT	WT
np20	WT	WT
nvdB	WT	WT
pelA	WT	WT
phnAB	WT	WT
phoB	WT	WT
phZ 1/2	WT	WT
phz1/pvdApchE	WT	WT
phzA1-G1 A2-G2	WT	WT
pqsA	WT	WT
pqsE	WT	WT
pqsH	WT	WT
pqsR	WT	WT
pvdA/pchE	WT	WT
pvdS	WT	WT
rhlA	High	WT
rhlB	High	WT
rhlC	WT	WT
rhlR	High	WT
roeA	WT	WT

rpoS	WT	WT
sadB	WT	WT
sadC	High	WT
sadC/roeA	WT	WT
spuF	WT	WT

[a]Viability of *S. constellatus* 7155 grown as mixed biofilms with the indicated strain of *P. aeruginosa* PA14 on plastic. “WT” indicates that the viability of *S. constellatus* 7155 was comparable to *S. constellatus* 7155 and wild-type *P. aeruginosa* PA14 mixed biofilms. “High” and ”Low” indicate greater or lesser viability of *S. constellatus* 7155 grown as mixed biofilms with the indicated strain of *P. aeruginosa* on plastic, respectively.

SUPPLEMENTAL MATERIAL 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:**1899-1902.
2. **Liberati NT, Urbach JM, Miyata S, Lee DG, Drenkard E, Wu G, Villanueva J, Wei T, Ausubel FM.** 2006. An ordered, nonredundant library of *Pseudomonas aeruginosa* strain PA14 transposon insertion mutants. Proc Natl Acad Sci U S A **103:**2833-2838.
3. **Kuchma SL, Brothers KM, Merritt JH, Liberati NT, Ausubel FM, O'Toole GA.** 2007. BifA, a cyclic-Di-GMP phosphodiesterase, inversely regulates biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. J Bacteriol **189:**8165-8178.
4. **Hogan DA, Vik A, Kolter R.** 2004. A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol Microbiol **54:**1212-1223.
5. **Cugini C, Morales DK, Hogan DA.** 2010. *Candida albicans*-produced farnesol stimulates *Pseudomonas* quinolone signal production in LasR-defective *Pseudomonas aeruginosa* strains. Microbiology **156:**3096-3107.
6. **Anderson GG, Yahr TL, Lovewell RR, O'Toole GA.** 2010. The *Pseudomonas aeruginosa* magnesium transporter MgtE inhibits transcription of the type III secretion system. Infect Immun **78:**1239-1249.
7. **Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA.** 2003. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature **426:**306-310.
8. **Friedman L, Kolter R.** 2004. Genes involved in matrix formation in *Pseudomonas aeruginosa* PA14 biofilms. Mol Microbiol **51:**675-690.
9. **Dietrich LE, Price-Whelan A, Petersen A, Whiteley M, Newman DK.** 2006. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. Mol Microbiol **61:**1308-1321.
10. **Ha DG, Merritt JH, Hampton TH, Hodgkinson JT, Janecek M, Spring DR, Welch M, O'Toole GA.** 2011. 2-Heptyl-4-quinolone, a precursor of the *Pseudomonas* quinolone signal molecule, modulates swarming motility in *Pseudomonas aeruginosa*. J Bacteriol **193:**6770-6780.
11. **Pukatzki S, Kessin RH, Mekalanos JJ.** 2002. The human pathogen *Pseudomonas aeruginosa* utilizes conserved virulence pathways to infect the social amoeba *Dictyostelium discoideum*. Proc Natl Acad Sci U S A **99:**3159-3164.
12. **Caiazza NC, Shanks RM, O'Toole GA.** 2005. Rhamnolipids modulate swarming motility patterns of *Pseudomonas aeruginosa*. J Bacteriol **187:**7351-7361.
13. **Hogan DA, Kolter R.** 2002. *Pseudomonas-Candida* interactions: an ecological role for virulence factors. Science **296:**2229-2232.
14. **Merritt JH, Ha DG, Cowles KN, Lu W, Morales DK, Rabinowitz J, Gitai Z, O'Toole GA.** 2010. Specific control of *Pseudomonas aeruginosa* surface-

associated behaviors by two c-di-GMP diguanylate cyclases. MBio **1**:pii: e00183-10.

15. **Caiazza NC, O'Toole GA.** 2004. SadB is required for the transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas aeruginosa* PA14. J Bacteriol **186:**4476-4485.
16. **Merritt JH, Brothers KM, Kuchma SL, O'Toole GA.** 2007. SadC reciprocally influences biofilm formation and swarming motility via modulation of exopolysaccharide production and flagellar function. J Bacteriol **189:**8154-8164.