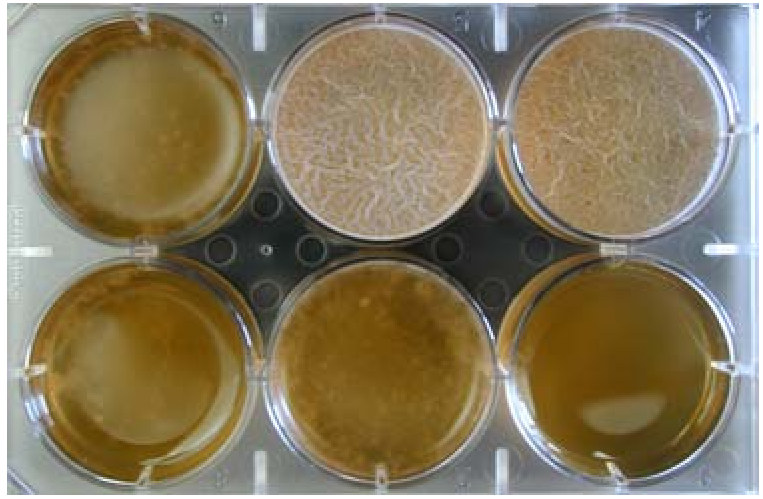
A

*sup*3 *sup*1



*sup*2

B

Δ*ywcC* Δ*sinI*

(Ala[28] → Glu) *sup*3

*sup*1

*sup*2

Figure S1

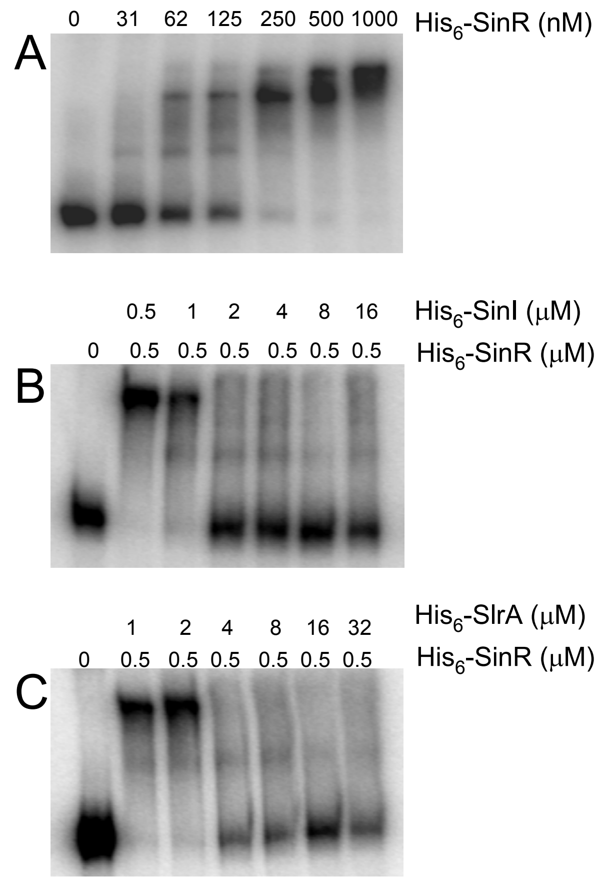

0 31 62 125 250 500 1000 His6-SinR (nM)
A
0.5 1 2 4 8 16 His6-SinI (μM)
0 0.5 0.5 0.5 0.5 0.5 0.5 His6-SinR (μM)
B
1 2 4 8 16 32 His6-SlrA (μM)
0 0.5 0.5 0.5 0.5 0.5 0.5 His6-SinR (μM)
C

Figure S2

Supplemental figure legends:

Figure S1. (A) In six independent inoculations of the Δ*ywcC* Δ*sinI* cells in 2X SGG medium, three out of six (*sup*1, *sup*2, *sup*3) cultures showed or began to show robust pellicle formation after three days of incubation at 23°C. (B) All three cultures contained suppressor mutants that are different from the parent strain and form robust biofilms. One such suppressor mutant (*sup*3) acquired a mutation that was mapped to the *sinR* gene as a missense mutation ($Ala^{28} \rightarrow$ Glu).

Figure S2. SlrA inhibits SinR from binding to DNA in EMSA (A) His_6-SinR bound to and shifted the promoter sequence of the *epsA-O* operon in a concentration dependent manner. Increasing amounts of His_6-SinI (as indicated in panel B) or His_6-SlrA (as indicated in panel C) were mixed with a fixed amount of His-SinR (0.5 μM) in EMSA. Note that the His-tagged SinI (and we presume the His-tagged SlrA) was less potent that the purified untagged SinI used in our previous EMSA experiments [Kearns et al (2005)].

Supplemental experimental procedures:

Electrophoretic mobility shift assay (EMSA)

Proteins were purified as described above. The DNA probe for the promoter sequence of the *eps* operon was generated by PCR using chromosomal DNA from 3610 and the primers P_{epsA}-F1 and P_{epsA}-R1 (Table S2). The DNA probe was digested with EcoRI, gel purified, and filled-in using klenow (*exo*$^{-}$), dTTP, and [α-32P]-dATP (NEB). EMSA was conducted following a protocol that has been described previously (Kearns et al., 2005).

Table S1. Strains used in this study.

E. coli		
DH5α	an *E. coli* strain used for molecular cloning	Invitrogen
BL21/DE3	*E. coli* B F$^-$ *dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3)	Stratagene
FC595	a BL21/DE3 derivative for overexpression of GST-SlrR, Cm^R, Amp^R	(FC, unpublished)
RL4219	a BL21/DE3 derivative for overexpression of His$_6$-SinI, Cm^R, Kan^R	(Kearns *et al.*, 2005)
RL4220	a BL21/DE3 derivative for overexpression of His$_6$-SinR, Cm^R, Kan^R	(Kearns et al., 2005)
YC388	a BL21/DE3 derivative for overexpression of His$_6$-SlrA, Kan^R	this work
B. subtilis		
PY79	laboratory strain used as a host for transformation	
3610	undomesticated wild strain capable of forming robust biofilms	(Branda et al., 2001)
RL3856	Δ*sinR*, Δ*epsH* in 3610, Spc^R, Tet^R	(Kearns *et al.*, 2005)
FC134	*amyE*::P$_{yqxM}$-*lacZ*, Δ*epsH* in 3610, Cm^R, Tet^R	(Chu *et al.*, 2008)
FC135	*amyE*::P$_{yqxM}$-*lacZ*, Δ*sinR,* Δ*epsH* in 3610, Cm^R, Spc^R, Tet^R	(Chu *et al.*, 2008)
YC122	*amyE*::P$_{slrR}$-*lacZ* in 3610, Cm^R	this work
YC130	*amyE*::P$_{epsA}$-*lacZ* in 3610, Cm^R	(Chai *et al.*, 2008)
YC131	Δ*slrR* in 3610, Spc^R	this work
YC132	*amyE*::P$_{epsA}$-*lacZ*, Δ*slrR* in 3610, Cm^R, Spc^R	this work
YC133	*amyE*::P$_{epsA}$-*lacZ*, Δ*sinR*, Δ*epsH* in 3610, Cm^R, Spc^R, Tet^R	this work
YC148	*amyE*::P$_{slrR}$-*lacZ*, Δ*slrR* in 3610, Cm^R, Spc^R	this work
YC189	*amyE*::P$_{yqxM}$-*cfp* in 3610, Spc^R	(Chai *et al.*, 2008)
YC274	*amyE*::P$_{yqxM}$-*lacZ*, Δ*slrR* in 3610, Spc^R, Tet^R	this work
YC294	Δ*slrA* in 3610, Kan^R	this work
YC295	Δ*ywcC* in 3610, Kan^R	this work
YC296	Δ*ywcC-slrA* in 3610, Kan^R	this work
YC297	Δ*ywcC*, Δ*slrR* in 3610, Kan^R, Spc^R	this work
YC298	Δ*ywcC*, Δ*sinI* in 3610, Kan^R, Spc^R	this work
YC501	*amyE*::P$_{yqxM}$-*lacZ*, Δ*slrA* in 3610, Cm^R, Kan^R	this work
YC502	*amyE*::P$_{epsA}$-*lacZ*, Δ*slrA* in 3610, Cm^R, Kan^R	this work
YC503	*amyE*::P$_{slrR}$-*lacZ*, Δ*slrA* in 3610, Cm^R, Kan^R	this work
YC505	*amyE*::P$_{yqxM}$-*lacZ*, Δ*ywcC*, Δ*epsH* in 3610, Cm^R, Kan^R, Tet^R	this work
YC506	*amyE*::P$_{epsA}$-*lacZ*, Δ*ywcC*, Δ*epsH* in 3610, Cm^R, Kan^R, Tet^R	this work
YC507	*amyE*::P$_{slrR}$-*lacZ*, Δ*ywcC*, Δ*epsH* in 3610, Cm^R, Kan^R, Tet^R	this work
YC509	*amyE*::P$_{yqxM}$-*lacZ*, Δ*ywcC-slrA* in 3610, Cm^R, Kan^R	this work
YC510	*amyE*::P$_{epsA}$-*lacZ*, Δ*ywcC-slrA* in 3610, Cm^R, Kan^R	this work
YC517	*amyE*::P$_{yqxM}$-*lacZ*, Δ*ywcC*, Δ*slrR* in 3610, Cm^R, Kan^R, Mls^R	this work
YC518	*amyE*::P$_{epsA}$-*lacZ*, Δ*ywcC*, Δ*slrR* in 3610, Cm^R, Kan^R, Mls^R	this work
YC519	*amyE*::P$_{slrR}$-*lacZ*, Δ*ywcC*, Δ*slrR,* Δ*epsH* in 3610, Cm^R, Kan^R, Spc^R, Tet^R	this work
YC526	*amyE*::P$_{slrA}$-*lacZ*, Δ*epsH* in 3610, Cm^R, Tet^R	this work
YC527	*amyE*::P$_{slrA}$-*lacZ*, Δ*ywcC*, Δ*epsH* in 3610, Cm^R, Kan^R, Tet^R	this work
YC528	*amyE*::P$_{yqxM}$-*lacZ*, Δ*slrR*, Δ*epsH* in 3610, Cm^R, Mls^R, Tet^R	this work
YC529	*amyE*::P$_{yqxM}$-*lacZ*, Δ*ywcC*, Δ*slrR*, Δ*epsH* in 3610, Cm^R, Kan^R, Mls^R, Tet^R	this work
YC530	Δ*ywcC-slrA*, Δ*slrR* in 3610, Kan^R, Mls^R	this work
YC531	*amyE*::P$_{slrR}$-*lacZ*, Δ*ywcC-slrA*, Δ*slrR* in 3610, Cm^R, Kan^R, Mls^R	this work
YC540	*amyE*::P$_{yqxM}$-*cfp*, Δ*ywcC*, Δ*epsH* in 3610, Spc^R, Kan^R, Tet^R	this work
YC563	*amyE*::P$_{slrA}$-*slrA*, Δ*slrA* in 3610, Cm^R, Kan^R	this work
YC564	*amyE*::P$_{slrA}$-*sinI*, Δ*slrA* in 3610, Cm^R, Kan^R	this work
YC567	*amyE*::P$_{slrA}$-*gfp* in 3610, Cm^R	this work
YC568	*amyE*::P$_{yqxM}$-*lacZ*, Δ*sinR*, Δ*slrA*, Δ*epsH* in 3610, Cm^R, Spc^R, Kan^R, Tet^R	this work
YC569	*amyE*::P$_{epsA}$-*lacZ*, Δ*sinR*, Δ*slrA*, Δ*epsH* in 3610, Cm^R, Spc^R, Kan^R, Tet^R	this work

Table S2. Primes used in this study.

P_{slrA}-F1:	5′-gtagaattcccctggcggtaatgattaccctt-3′
P_{slrA}-R1:	5′-gtaggatccaattgtataaaatgttttcctg-3′
P_{slrA}-R2:	5′-gtacatatgaacctccaattgtataaaatgt-3′
P_{slrR}-F1:	5′-gtagaattcctagacaatcgcatataattctttg-3′
P_{slrR}-R1:	5′-gtcggatccctagaaattctcctctattcctgtcg-3′
P_{epsA}-F1:	5′-gtcgaattcctagaaattctcctctattcctgtcg-3′
P_{epsA}-R1:	5′-gtcgaattcctagacaatcgcatataattctttg–3′
sinI-F2:	5′-gtacatatgaagaatgcaaaacaagagcac-3′
sinI-R2:	5′-gtaggatcctcagaaaggatttacggtatg-3′
slrA-R1:	5′-gtaggatccctagtcttgccggacggttttt-3′
ywcC-P1:	5′-gacgccgataaaatggttttccg-3′
ywcC-P2:	5′-caattcgccctatagtgagtcgttcagtgaagtatagagaaata-3′
ywcC-P3:	5′-ccagcttttgttccctttagtgagatttttctcctggcggtaatg-3′
ywcC-P4:	5′-caataaaagcgcgtttctgctt -3′
slrA-P1:	5′-gagagtgcgtctaaaaagctgcg-3′
slrA-P2:	5′-caattcgccctatagtgagtcgttcatagtaacctccaattgta-3′
slrA-P3:	5′-ccagcttttgttccctttagtgagaagactagtccgaacaggcgg-3′
slrA-P4:	5′-gatgtacaagacaacgagataag -3′