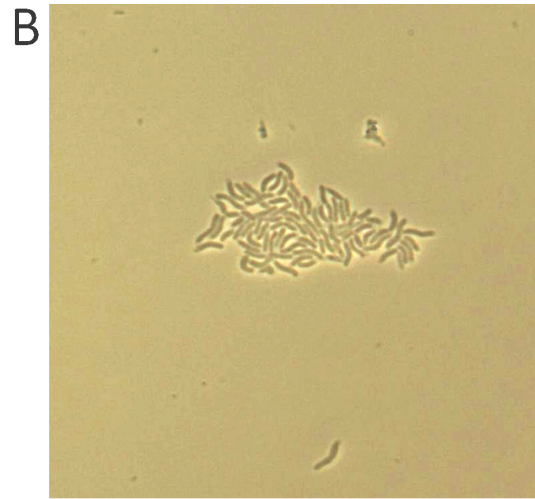
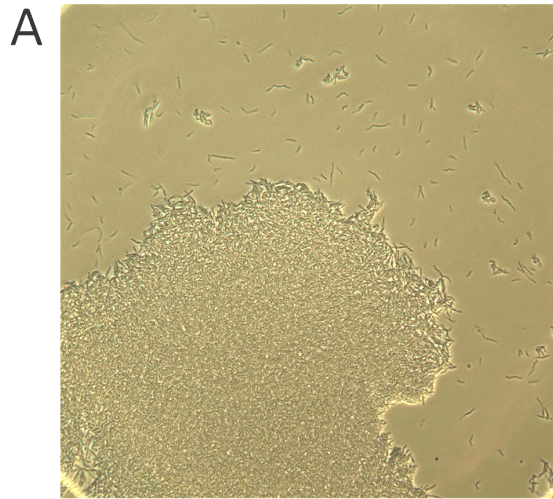


1

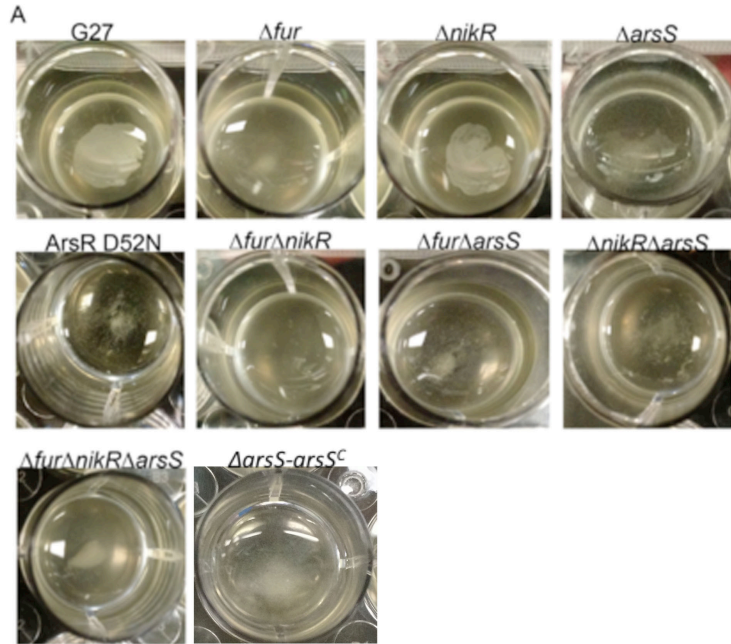


2

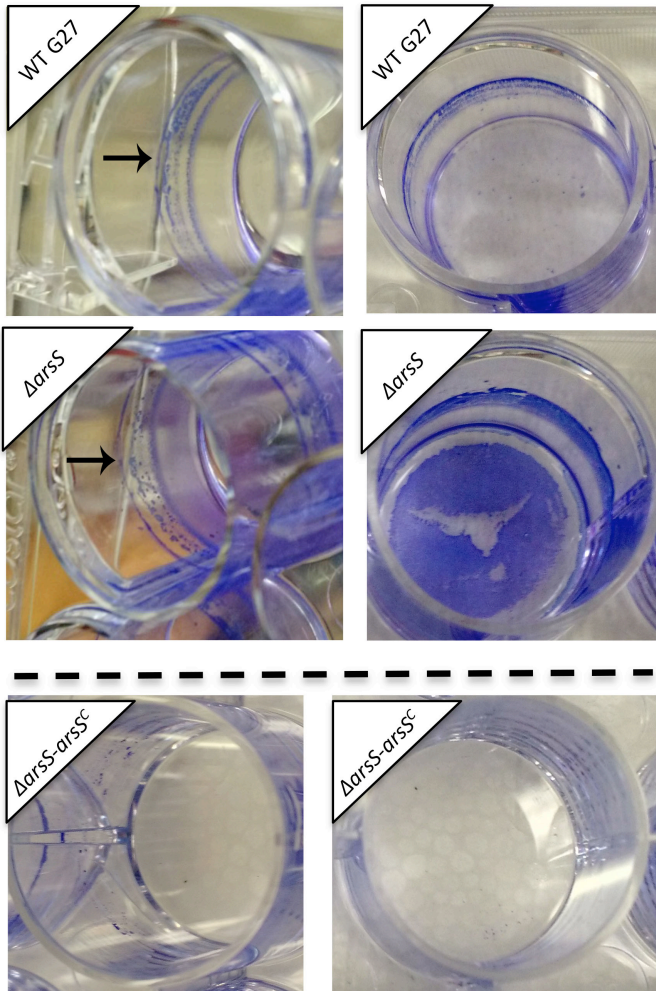
3 **Supplemental Figure 1.**

4 Phase contrast images of the $\Delta arsS$ mutant strain were taken after 72 hours of

5 growth. **A.** 40X magnification and **B.** 100x oil emersion.



B



7 **Supplemental Figure 2.**

8 **A.** Representative images of the various biofilm phenotypes observed after 72

9 hours of growth in 24 well plates. The strains grown in each well are labeled.

10 Wild-type G27 and $\Delta nikR$ both exhibit pellicle formation in addition to biofilm

11 formation at the air-liquid interface, Δfur and $\Delta fur\Delta nikR$ display biofilm

12 formation only at the air-liquid interface, and strains lacking $\Delta arsS$ or

13 containing the *ArsR*-D52N mutation appear granular due to auto-aggregation

14 and form biofilm at the air-liquid interface and on the bottom of the well. The

15 $\Delta arsS-arsS^C$ exhibited slight biofilm formation at the liquid-air interface as well

16 as minimal aggregation.

17 **B.** Representative images of G27 and the $\Delta arsS$ strain after crystal violet staining

18 of 72 hour cultures. Both strains show prominent staining at the air-liquid

19 interface; however, the $\Delta arsS$ mutant strain also shows significant staining

20 along the side and bottom of the well (arrow). Below the dashed line are

21 representative images from the $\Delta arsS-arsS^C$ biofilm assays; complementation

22 resulted in a loss of the hyper-biofilm phenotype.

23

24

25

Table S1. Comparison of the degree of biofilm formation at each time point

T24	Adjusted P Value [#]	Summary
G27 vs. G27 Δ arsS	0.0008	** ₁ ,A
G27 vs. G27 Δ fur Δ arsS	0.0231	** ₁ ,A
G27 vs. G27 Δ nikR Δ arsS	0.005	* ₁ ,A
G27 vs. G27 Δ fur Δ nikR Δ arsS	0.004	***
G27 Δ fur vs. G27 Δ arsS	0.002	***
G27 Δ fur vs. G27 Δ fur Δ arsS	0.0447	***
G27 Δ fur vs. G27 Δ nikR Δ arsS	0.0107	** ₁ ,A
G27 Δ fur vs. G27 Δ fur Δ nikR Δ arsS	0.0086	***
G27 Δ nikR vs. G27 Δ arsS	0.0003	***
G27 Δ nikR vs. G27 Δ fur Δ arsS	0.0091	***
G27 Δ nikR vs. G27 Δ nikR Δ arsS	0.0018	** ₁ ,A
G27 Δ nikR vs. G27 Δ fur Δ nikR Δ arsS	0.0014	***
G27 Δ fur Δ nikR vs. G27 Δ arsS	0.0005	***
G27 Δ fur Δ nikR vs. G27 Δ fur Δ arsS	0.0144	***
G27 Δ fur Δ nikR vs. G27 Δ nikR Δ arsS	0.003	** ₁ ,A
G27 Δ fur Δ nikR vs. G27 Δ fur Δ nikR Δ arsS	0.0023	***
48	Adjusted P Value [#]	Summary
G27 vs. G27 Δ arsS	< 0.0001	** ₁ ,A
G27 vs. G27 Δ fur Δ arsS	0.0001	** ₁ ,A
G27 vs. G27 Δ fur Δ nikR Δ arsS	0.0004	***
G27 vs. ArsR D52N	< 0.0001	** ₁ ,B
G27 Δ fur vs. G27 Δ arsS	< 0.0001	***
G27 Δ fur vs. G27 Δ fur Δ arsS	< 0.0001	***
G27 Δ fur vs. G27 Δ nikR Δ arsS	0.0366	** ₁ ,A
G27 Δ fur vs. G27 Δ fur Δ nikR Δ arsS	0.0001	***
G27 Δ fur vs. ArsR D52N	< 0.0001	** ₁ ,B
G27 Δ nikR vs. G27 Δ arsS	< 0.0001	***
G27 Δ nikR vs. G27 Δ fur Δ arsS	< 0.0001	***
G27 Δ nikR vs. G27 Δ nikR Δ arsS	0.0017	** ₁ ,A
G27 Δ nikR vs. G27 Δ fur Δ nikR Δ arsS	< 0.0001	***
G27 Δ nikR vs. ArsR D52N	< 0.0001	** ₁ ,B
G27 Δ fur Δ nikR vs. G27 Δ arsS	< 0.0001	***
G27 Δ fur Δ nikR vs. G27 Δ fur Δ arsS	< 0.0001	***
G27 Δ fur Δ nikR vs. G27 Δ nikR Δ arsS	0.0149	** ₁ ,A
G27 Δ fur Δ nikR vs. G27 Δ fur Δ nikR Δ arsS	< 0.0001	***
G27 Δ fur Δ nikR vs. ArsR D52N	< 0.0001	** ₁ ,B
72	Adjusted P Value [#]	Summary
G27 vs. G27 Δ fur Δ nikR Δ arsS	0.0162	***

G27 vs. ArsR D52N	0.0199	** ^{,B}
G27 Δ fur vs. G27 Δ arsS	0.0024	***
G27 Δ fur vs. G27 Δ fur Δ arsS	0.0115	***
G27 Δ fur vs. G27 Δ fur Δ nikR Δ arsS	0.0005	***
G27 Δ fur vs. ArsR D52N	0.0006	** ^{,B}
G27 Δ nikR vs. G27 Δ arsS	0.0016	***
G27 Δ nikR vs. G27 Δ fur Δ arsS	0.008	***
G27 Δ nikR vs. G27 Δ fur Δ nikR Δ arsS	0.0003	***
G27 Δ nikR vs. ArsR D52N	0.0004	** ^{,B}
G27 Δ fur Δ nikR vs. G27 Δ arsS	0.0004	***
G27 Δ fur Δ nikR vs. G27 Δ fur Δ arsS	0.002	***
G27 Δ fur Δ nikR vs. G27 Δ fur Δ nikR Δ arsS	< 0.0001	***
G27 Δ fur Δ nikR vs. ArsR D52N	< 0.0001	** ^{,B}
G27 Δ nikR Δ arsS vs. G27 Δ fur Δ nikR Δ arsS	0.0417	* ^{,B}

2-way ANOVA with Tukey's multiple comparisons test

*^{,A} significant 24 hours

*^{,B} significant at 72 hours

**^{,A} significant at 24 and 48 hours

**^{,B} significant at 48 and 72 hours

*** significant 24, 48, and 72 hours