**SUPPORTING INFORMATION**

# A Complex LuxR-LuxI Type Quorum Sensing Network in a Roseobacterial Marine Sponge Symbiont Activates Flagellar Motility and Inhibits Biofilm Formation

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## SUPPLEMENTARY METHODS

**TLC profiling of AHLs.** Five ml MB 2216 cultures from KLH11 were grown to an $OD_{600}$ of 1.5 – 2.0, followed by extraction with an equal volume of dichloromethane. Culture pH was monitored and was within the range of 7.6±0.2 at the time of harvesting (sterile Marine Broth 2216 is pH 7.6). Following centrifugation, the organic phase was removed and allowed to evaporate in a fume hood. Extracts were concentrated 1000 fold and normalized to an $OD_{600}$ of 1.5 and resuspended in a final volume of approximately 5 µl of acidified (0.01%) ethyl acetate and loaded onto a C18 RP-TLC plate (Mallinckrodt Baker, Phillipsburg, NJ, USA). TLC plates were developed in a 60% methanol water mobile phase, dried, and overlaid with 100 ml of 0.6% ATGN media supplemented with 40 µg $ml^{-1}$ X-gal and 1 ml of an $OD_{600}$ = 12.0 suspension of the highly sensitive *A. tumefaciens* AHL reporter (*A. tumefaciens* KYC55 [pJZ372][pJZ384][pJZ410]) as previously described (Zhu *et al.*, 2003). TLC plate overlays were placed in a sealed container and incubated at 28˚C for 16-48 hrs.

**Derivatization of AHL samples.** The formation of the methoxylamine derivatives of the AHL samples (MC4100 expressing SsaI or SsbI) was carried out using 0.5 ml of methoxylamine HCL (1.2 M) sodium acetate trihydrate (200 mM) in 87.5% methanol and 12.5% distilled $H_2O$ followed by 1 h incubation at 37˚C, then diluted with 2 ml H2O. This diluted reaction solution was passed through a pre-activated C18 reversed solid phase extraction cartridge. The sample was eluted with 1 ml methanol, taken to dryness and resuspended in 30 ul of 95% solvent A 5% solvent B and analyzed as

positive ions by LC/MS. The methoxylated AHLs were found to elute approximately 3 min later than un-derivatized AHLs and had a molecular weight increase by 29 u. The trimethylsilylation of the equivalent AHL samples was carried out by adding equal parts of bis(trimethylsily)trifluoroacetamide and acetonitrile to a dried aliquot of the sample for a total volume of 50 µl, followed by incubating at 60˚C 1 h in a capped culture tube. The reacted product was taken to dryness and resuspended in 30 µl of 95% solvent A 5% solvent B and analyzed as positive ions using the same LC/MS/MS conditions. Trimethylsilated derivatives were retained on the column 5.2 min later than un-derivatized AHLs and had a molecular weight increase by 72 u.

**Immunoblotting with anti-flagellar antibodies.** Samples were separated by 15% SDS-PAGE at 100V 80-90 min, and transferred to the membrane (Osmonics, Westborough, MA) at 30V for 40 min using a semi-dry electrotransfer system. The membrane was blocked overnight at 4°C in Blotto (1x TBS-T [Tris Buffered Saline], 1% Tween 20) and 5% dried milk). The polyclonal antibody was diluted 1:20,000 in 4 ml of Blotto and incubated with the membrane for 45 min on a rocking shaker at room temperature. The membrane was then washed three times for 5 min in 1X TBS-T. Secondary antibody solution at 1:20,000 in 4 ml of Blotto was incubated with the membrane and rocked 45 min at room temp. The blot was rinsed three times for 5 min with 1X TBS-T, followed by 2 times in 1X TBS. Chemoluminescent substrate, Supersignal West Pico Chemoluminescent Substrate (Pierce, Rockford, IL) was used as the detection reagent. Equal parts of the Luminol/Enhancer and Stable Peroxide Buffer were combined and pooled on top of the membrane for ~5 min. Excess detection

reagent was removed by blotting on filter paper, and signal was detected by exposure to Kodak BioMax film (Kodak).

## SUPPLEMENTARY TABLES

**Supplementary Table 1. Cross-regulation experiments for SsaR and SsbR in an AHL$^-$ host[1]**

| | | β-Galactosidase Sp. Act.[2] | |
|---|---|---|---|
| **Expression plasmid** | **Fusion plasmid** | **No AHL** | **+ AHL[3]** |
| Vector (pBBR1-MCS5) | *ssbI-lacZ* (pEC121) | 0.7 (0.1) | 0.8 (0.1)) |
| $P_{lac}$-*ssaR* (pEC112) | *ssbI-lacZ* (pEC121) | 0.9 (0.1) | 0.9 (0.2) |
| Vector (pBBR1-MCS5) | *ssaI-lacZ* (pEC116) | 57.9 (0.9) | 58.5 (3.6) |
| $P_{lac}$-*ssbR* (pEC123) | *ssaI-lacZ* (pEC116) | 50.6 (3.1) | 44.3 (2.8) |

[1] All strains derived from Ti-plasmidless *A. tumefaciens* NTL4

[2] Specific activity in Miller Units, averages of assays in triplicate (standard deviation)

[3] 2 μM 3-oxo-C16:1 Δ11-HSL was added for $P_{lac}$-*ssaR* and 20 μM 3-OH-C14-HSL was added for $P_{lac}$-*ssbR.*

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**Supplementary Table S2. Relevant Strains and Plasmids**

| Bacteria/Plasmid | Relevant feature[b] | Reference |
|---|---|---|
| *E. coli* Electro-Ten Blue | Standard alpha-complementation strain | Stratagene |
| *E. coli* DH5α/λpir | Strain for propagating R6K suicide plasmids | Lab collection |
| *E. coli* S17-1/λpir | IncP conjugal donor | (Kalogeraki and Winans, 1997) |
| *E.coli* XL-1 Blue | Standard alpha-complementation strain | Lab collection |
| *E. coli* TOP 10 F´ | Standard alpha-complementation strain, *lacI*$^{Q}$ | Qiagen |
| *E. coli* MC4100 | K-12 derivative, Δ*lacZ* | (Casadaban, 1976) |
| *A. tumefaciens* NTL4 | Ti plasmidless derivative, nopaline chromosomal background | (Zhu *et al.*, 1998) |
| *A. tumefaciens* KYC55 | Ti plasmidless derivative, octopine chromosomal background | (Cho *et al.*, 1997; Zhu *et al.*, 2003) |
| *Ruegeria pomeroyi* DSS-3 | wild type | (Gonzales *et al.*, 2003) |
| KLH11 | wild type | (Mohamed *et al.*, 2008) |
| KLH11-EC1 | Rif$^{R}$ | This study |
| KLH11-EC2 | *ssaI-lacZ,* null *ssaI*, Rif$^{R}$, Km$^{R}$ | This study |
| KLH11-SK01 | Δ*ssaI,* Rif$^{R}$ | This study |
| KLH11-EC3 | *ssbI-lacZ,* null *ssbI*, Rif$^{R}$, Km$^{R}$ | This study |
| KLH11-SK02 | Δ*ssaI* Δ*ssbI* Rif$^{R}$ | This study |
| KLH11-EC4 | *ssaR-lacZ,* null *ssaR,* Rif$^{R}$, Km$^{R}$ | This study |
| KLH11-EC5 | *ssbR-lacZ,* null *ssbR* Rif$^{R}$, Km$^{R}$ | This study |
| KLH11-JZ1 | *ssaR-lacZ,* wild type *ssaR,* Rif$^{R}$, Km$^{R}$ | This study |
| KLH11-JZ2 | *ssbR-lacZ,* wild type *ssbR,* Rif$^{R}$, Km$^{R}$ | This study |
| pCR®2.1-TOPO® | PCR fragment cloning vector, Ap/Km$^{R}$ | Invitrogen |

| | | |
|---|---|---|
| pBBR1-MCS2 | $P_{lac}$ expression vector, $Km^R$ | (Kovach *et al.*, 1995) |
| pBBR1-MCS5 | $P_{lac}$ expression vector, $Gm^R$ | (Kovach *et al*., 1995) |
| pGEM®T-Easy | PCR fragment cloning vector, $Ap^R$ | Promega |
| pVIK112 | R6K-based *lacZ* transcriptional fusion, integration vector, $Km^R$ | (Kalogeraki and Winans, 1997) |
| pRA301 | *lacZ* translational fusion vector | (Akakura and Winans, 2002) |
| pJZ372 | $P_{traI}$-*lacZ* translation fusion, $Tet^R$ | (Zhu *et al*., 2003) |
| pJZ384 | $P_{T7::}$*traR*, $Sp^R$ | (Zhu *et al*., 2003) |
| pJZ410 | T7 polymerase expressing plasmid | (Zhu *et al*., 2003) |
| pECH100 | pBBR1-MCS5 derivative, 3 kb *Hin*D III fragment containing *ssaI* and truncated *ssaR*, $Gm^R$ | This study |
| pECH101 | pBBR1-MCS5 derivative, 2.8 kb *Hin*D III fragment containing *ssbI* and truncated *ssbR,* $Gm^R$ | This study |
| pECS102 | pBBR1-MCS5 derivative, 3.2 kb *Sal* I fragment containing *ssaI* and *ssaR*, $Gm^R$ | This study |
| pEC103 | pGEM®T-Easy derivative, carrying truncated *ssaI* fragment, $Ap^R$ | This study |
| pEC104 | pGEM®T-Easy derivative, carrying truncated *ssbI* fragment, $Ap^R$ | This study |
| pEC105 | pGEM®T-Easy derivative, carrying truncated *ssaR* fragment, $Ap^R$ | This study |
| pEC106 | pGEM®T-Easy derivative, carrying full length PCR-amplified *ssbR* fragment, $Ap^R$ | This study |
| pEC107 | pVIK112 derivative carrying truncated *ssaR* gene from pEC105, $Km^R$ | This study |

| | | |
|---|---|---|
| pEC108 | pBBR1-MCS5 derivative carrying full length $P_{lac}$-*ssaI*, from pEC111, $Gm^R$ | This study |
| pEC109 | pBBR1-MCS5 derivation carrying full length $P_{lac}$-*ssbI*, from pEC110, $Gm^R$ | This study |
| pEC110 | pCR®2.1-TOPO® derivative carrying full length PCR-amplified $P_{lac}$-*ssbI*, Ap/$Km^R$ | This study |
| pEC111 | pCR®2.1-TOPO® derivative carrying full length PCR-amplified $P_{lac}$-*ssaI*, Ap/$Km^R$ | This study |
| pEC112 | pBBR1-MCS5 derivative, carrying full length $P_{lac}$-*ssaR*, from pEC106, $Gm^R$ | This study |
| pEC113 | pVIK112 derivation carrying truncated *ssaI* gene from pEC103, $Km^R$ | This study |
| pEC114 | pCR®2.1-TOPO® derivative, carrying PCR amplified $P_{ssaI}$, Ap/$Km^R$ | This study |
| pEC115 | pVIK112 derivative carrying truncated *ssbI* gene from pEC104, $Km^R$ | This study |
| pEC116 | pRA301 derivation, $P_{ssaI}$-*lacZ*, $Sp^R$ | This study |
| pEC117 | pGEM®T-Easy derivative, carrying $P_{ssbR}$ and *ssbR* $Ap^R$ | This study |
| pEC118 | pGEM®T-Easy derivative, carrying $P_{ssbI}$ and *ssbI* $Ap^R$ | This study |
| pEC119 | pGEM®T-Easy derivative, carrying $P_{ssbR}$ and *ssbR,* $Ap^R$ | This study |
| pEC120 | pGEM®T-Easy derivative, carrying full length $P_{lac}$-*ssbR*, $Ap^R$ | This study |
| pEC121 | pRA301 derivative, $P_{ssbI}$-*lacZ*, Sp/$Sm^R$ | This study |
| pEC122 | pVIK112 derivative carrying truncated *ssbR* from pEC119, $Km^R$ | This study |
| pEC123 | pBBR1-MCS5 derivative, carrying full length $P_{lac}$-*ssbR*, from pEC120, $Gm^R$ | This study |

| | | |
|---|---|---|
| pEC124 | pRA301 derivative, $P_{ssaI}$-*lacZ*, 5' promoter deletion, 79 bp with *lux* type box, $Sp^R$ | This study |
| pEC127 | pRA301 derivative, $P_{ssaI}$-*lacZ*, 5' promoter deletion, 63 bp fragment lacks *lux* type box, $Sp^R$ | This study |
| pJZ001 | pVIK112 derivative, *ssaR* gene with 5' truncation, to retain wt *ssaR*, $Km^R$ | This study |
| pJZ002 | pVIK112 derivative, *ssbR* gene with 5' truncation, to retain wt *ssbR*, $Km^R$ | This study |
| | | |

[b] Ap = Ampicillin, Gm=Gentamycin, Km=Kanamycin, Rif=Rifampcin, Sp=Spectinomycin. Tc=Tetracycline

**Supplementary Table S3. Primers used in this study.**

| Primer name | Sequence[a] (5'-3') | Restriction Enzyme |
|---|---|---|
| ssaI D1 | ACTAGTCTATGGTGACGACTGGAAG | SpeI |
| ssaI D2 | **GAATTCGTCAGTCAGTCA**GTTTCCCGTAATATTGGCTT | NA |
| ssaI D3 | **TGACTGACTGACGAATTC**AGGCTGGCGAACTCAAGCCTG | NA |
| ssaI D4 | GCATGCGACTACATTGTCGAGCTG | SphI |
| ssbI D1 | ACTAGTGCAATCAGGGTTATTCGATC | SpeI |
| ssbI D2 | **GAATTCGTCAGTCA**GTCACAACATGATTGTTCCCCTTGT | NA |
| ssbI D3 | **TGACTGACTGACGAATTC**GCCTGACCTTGGTGGAAATTG | NA |
| ssbI D4 | GCATGCGATACGGTGAATGGTCGTTGC | SphI |
| ssaI 1 | cggGAATTCATGTTCGAACTGCGCGCTCGGG | EcoRI |
| ssaI 2 | gccGGTACCATCGCAGGGACCTTGCCCATC | KpnI |
| ssaI 3 | ggcCTCGAGC**TGA**AAC*AGGAAA*CAGCT**ATG**ATTTTGGTAGTTGATG | XhoI |
| ssaI 4 | ggcGAATTCGGG**TCA**GGCCTCATGAGCAAAAGC | EcoRI |
| ssaR 1 | cgcGAATTCTCAGCACCCTCCCCGAACAGG | EcoRI |
| ssaR 2 | cgcGGTACCCGGCCCATTGCAAAATCTC | KpnI |
| ssaR 3 | gggCTCGAGG**TGA**AAC*AGGAAA*CAGCT**ATG**GATATTGTTGATCTCAGC | XhoI |
| ssaR 4 | gggGAATTCGGC**TTA**ACCTGGGTAGATTAGCCC | EcoRI |
| ssbI 1 | cgcGAATTCGGATGAGCTGCATAAATTTCCGG | EcoRI |
| ssbI 2 | gccGGTACCAACGGAAATCCGGTCTCGCCCG) | KpnI |
| ssbI 3 | ggcCTCGAGC**TGA**AAC*AGGAAA*CAGCT**ATG**TTGCGTTATTTATATGCGG | XhoI |
| ssbI 4 | ggcGAATTCGGG**TCA**GGCGGAAAGCGCAAACCG | EcoRI |
| ssbR 1 | ggcGAATTCTCGGTTCAGCTGCGCGATCGG | EcoRI |
| ssbR 2 | cgcGGTACCTTCCGGTTGCCGGTTCTGGGC | KpnI |
| ssbR 3 | gcgCTCGAGG**TGA**AACAGGAAAcagctATGAGGCTTGCGCGCCCGCG | XhoI |
| ssbR 4 | gggGAATTCGGC**TTA**AACGACTATTAATCCTCTGC | EcoRI |
| fliC 1 | cgcgaattcAAGTCGGTCAACATGAACCTG | EcoRI |
| fliC 2 | gccggtaccGTTGTCACGATCAAGCGAGGA | KpnI |
| fliCRT1 | CGCAGAACCTGTCGACCGGT | NA |
| fliCRT2 | GGTATCGCCTGCGGCCAATGT | NA |

| | | |
|---|---|---|
| flaART1 | GCACCGATGCATATGCGCAAGCT | NA |
| flaART2 | TGAGCGATTGCAGCCGGGTT | NA |
| rpoDRT1 | GACGCCTATCGCGGCCGT | NA |
| rpoDRT2 | GCCGACCTGCGCCATATCGT | NA |
| ssaI P1 | ccgGAATTCTGCACTAACCACACCTCAGGCCG | EcoRI |
| ssaI P2 | gcgGAATTCTACGGGAAACCCCCAATAGATTCG | EcoRI |
| ssaI P3 | gggGAATTCTAGATTCGCTGTGAAATCCGAG | EcoRI |
| ssaI P4 | ccgCTGCAGAAT**CAT**GTTAACCCCCTTCG | PstI |
| ssbI P1 | cggGAATTCTATAGCCGGGCACAGGTGGCGC | EcoRI |
| ssbI P2 | cccCTGCAGCAA**CAT**GATTGTTCCCCTTGTCGT | PstI |
| aP1 | CGGCACCATTCATGGCCATGT | NA |
| aP2 | CCATTCGTCCCGACTGCAGC | NA |
| aP3 | ATGATTTTGGTAGTTGATG | NA |
| aP4 | GTCGCATAGGACACCGAGTT | NA |
| bP1 | CCAATATGGCTTCACGACCT | NA |
| bP2 | AATAACCCTGATTGCCCACA | NA |
| bP3 | GGTAAACGAAGATGGCGAAG | NA |
| bP4 | GAGCCGATCATGCGATAAAT | NA |
| ssaRintactF | GAATTCCAAGGCCTGCATCTGATCG | EcoRI |
| ssaRintactR | GGTACC**TTA**ACCTGGGTAGATTAGCCC | KpnI |
| ssbRintactF | GAATTCTATCACCGCATTGATCCGG | EcoRI |
| ssbRintactR | GGTACC**TTA**AACGACTATTAATCCTCTGCTG | KpnI |
| RtaF | AAGTACTTGACGAAATGTTCGAACTG | NA |
| RtaR | GGTCGATCACGGTAATGATGTCTTC | NA |

[a] Engineered restriction sequences are underlined. Complementary sequences for PCR-SOEing are shown in bold and are also underlined. Start and stop codons are in bold. *E. coli lacZ* ribosomal binding sites are in italics and bold. Protection nucleotides for restriction enzyme are shown in lower case. *NA*= not applied.

## Supplementary References


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## SUPPLEMENTARY FIGURE LEGENDS

**Figure S1**. **Comparative analysis of AHLs.** The relative amounts of the known AHLs in (A and C) KLH11 derivatives and (B) E. coli MC4100 are shown in bar graphs, with the peak label above each set of bars. The plot shows the ratio of the area of the transition for each AHL to m/z 102 to the same transition for the internal standard d3-C6-HSL. Analysis of AHL samples was performed in positive-ion mode with the third quadrupole set to monitor m/z 102.3 as described in Experimental Procedures. The relative amounts of the known AHLs in each strain are shown in the bar graph, and the data were analyzed as in Figures 2 and 3. (e), (f), (g), and (h) referring to detection of 3OH-C12-HSL, 3OH-C13-HSL/3OH-C14:1-HSL, 3OH-C14-HSL/3oxoC14-HSL, and 3oxoC16-HSL/3OHC16:1-HSL respectively. In panels B and C, the SsaI y axis is on the left and the SsbI y axis (with an asterisk) is on the right.

**Figure S2. Gene maps of KLH11 *ssaR/ssaI* and *ssbR/ssbI* loci.** Arrows represent genes. A) SsaR (ZP_05123091) and SsaI (ZP_05123801) and are predicted to be 233 and 284 amino acids, respectively; Genbank accession numbers are ZP_05124568 for transglycosylase and ZP_05123238 for trigger factor. Primers used to test whether *ssaRI* are in the same operon are indicated as P1, P2, P3 and P4 (see Table S3, aP1, aP2, aP3, aP4). Lanes 1-4 were PCR results using primers P1 and P2, lanes 5-8 used primers P3 and P4, and lanes 9-12 used primers P1 and P4. Lanes 1, 5 and 9 used cDNA as template, lanes 2, 6 and 10 used genomic DNA as template, lanes 3, 7 and 10 used RNA as templates. Lanes 4, 8 and 12 were negative controls the primer sets. B)

SsbR (ZP_05123460) and SsbI (ZP_05121795) are predicted to be 239 and 212 amino acids. ; Genbank accession numbers are ZP_05124465 for crotonyl CoA reductase and ZP_05122236 for helicase. Primers used to test whether ssbRI are in the same operon are indicated as P1, P2, P3 and P4 (see Table S3, primers bP1, bP2, bP3, bP4). The lanes are organized the same as in panel A.

**Figure S3. Sequence alignment of selected AHL synthases.** The grey shaded regions are the most conserved sequence blocks within the AHL synthase family. Residues are colored red to indicate acidic or hydrophilic, blue for basic, and orange for other. Shaded residues are absolutely conserved and the boxed residues are the most similar regions within the family.

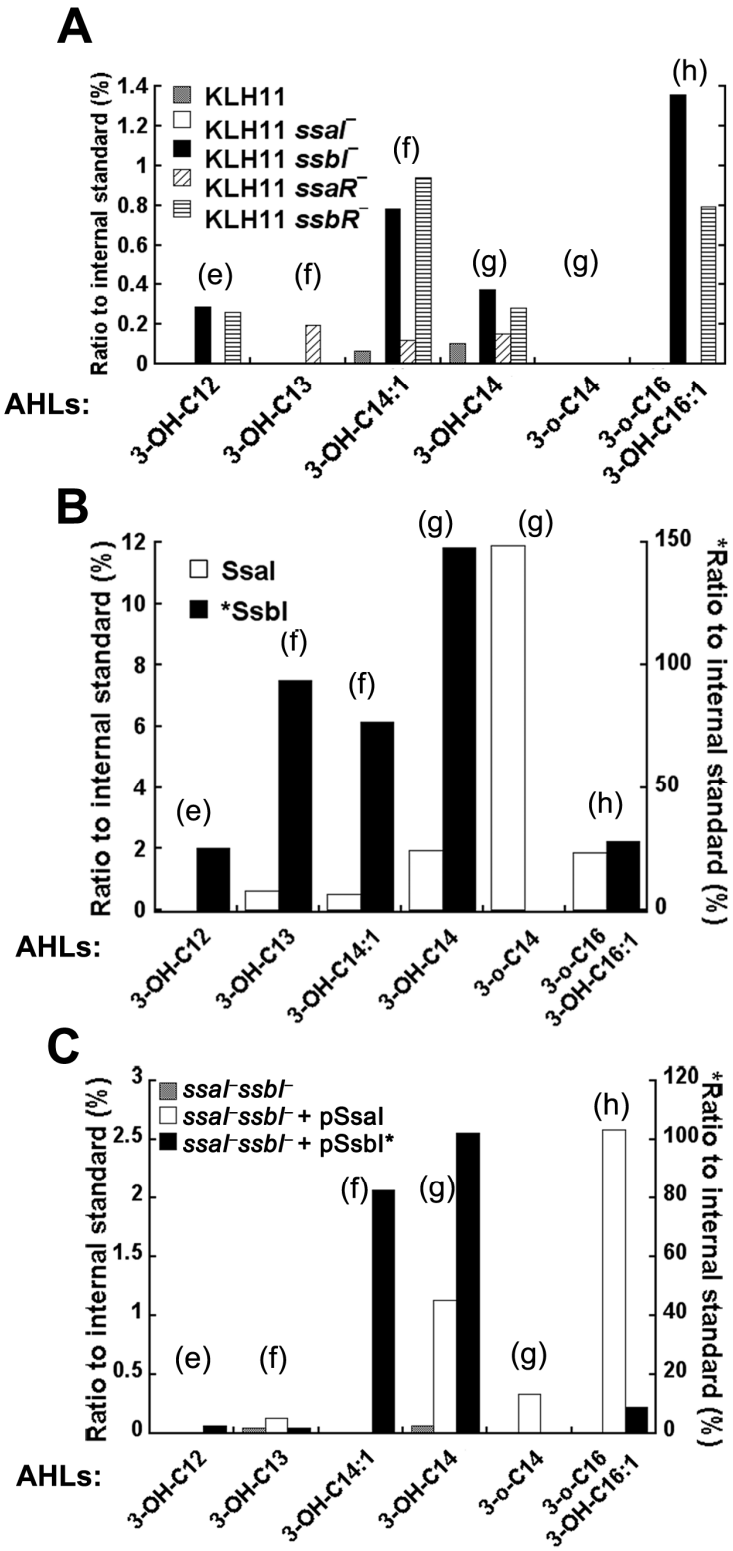
**Figure S4. Mass spectrometric analysis of plasmid-expressed SscI-directed AHLs from E. coli.** Cultures of E. coli MC4100 expressing plasmid-borne sscI were extracted and subjected to reverse-phase chromatographic separation prior to tandem MS analysis using the precursor ion-scanning mode (transitions were monitored for precursor [M + H]+ -> m/z 102) for (A) MC4100 + SscI. The peaks in the chromatograms are labeled with lettering and the AHLs are noted. (B) The relative amounts of the known AHLs in E. coli MC4100 derivatives harboring Plac expression plasmids with SsaI, SsbI, and SscI are shown in bar graphs, with the peak label above each set of bars. The plot shows the ratio of the area of the transition for each AHL to m/z 102 to the same transition for the internal standard D3-C6-HSL. The SsbI/SscI y axis is on the left, and the SsaI y axis is on the right.

**Figure S5**. **Activation of *ssaI* in response to synthetic AHLs** (A) Activation of *ssaI-lacZ* fusion with synthetic long chain AHLs. KLH11 *ssaI-* carrying the integrated *ssaI-lacZ* fusion. The final concentration of 3-OH-C14 used was 20 μM and the other 3 AHLs were 2 μM (B) Dose responsive activation of *ssaI-lacZ* by SsaR ($P_{lac}$-*ssaR*) in *A. tumefaciens* NTL4 background. Different concentrations of the crude organic extract of KLH11 cultures (%, v/v) and 3-oxo-C16:1 Δ11 (nM) were added at the time of culture inoculation. Miller Units are averages of assays performed in triplicate and error bars are standard deviations.

**Figure S6**. **Growth curves of KLH11 and different QS mutants.** Average OD600 of 5 ml cultures in MB2216. Cultures were grown in triplicate.

**Figure S7. Flagellar staining of KLH11 quorum-sensing mutants.** Stained cells from late stage cultures were viewed under phase contrast microscopy with 100X lens. Wild type (EC1), *ssaI-* (EC2), *ssbI-* (EC3), *ssaR-* (EC4), and *ssbR-* (EC5). Red arrows indicate stained flagella.

**Zan et al., Supplementary Figure 1**

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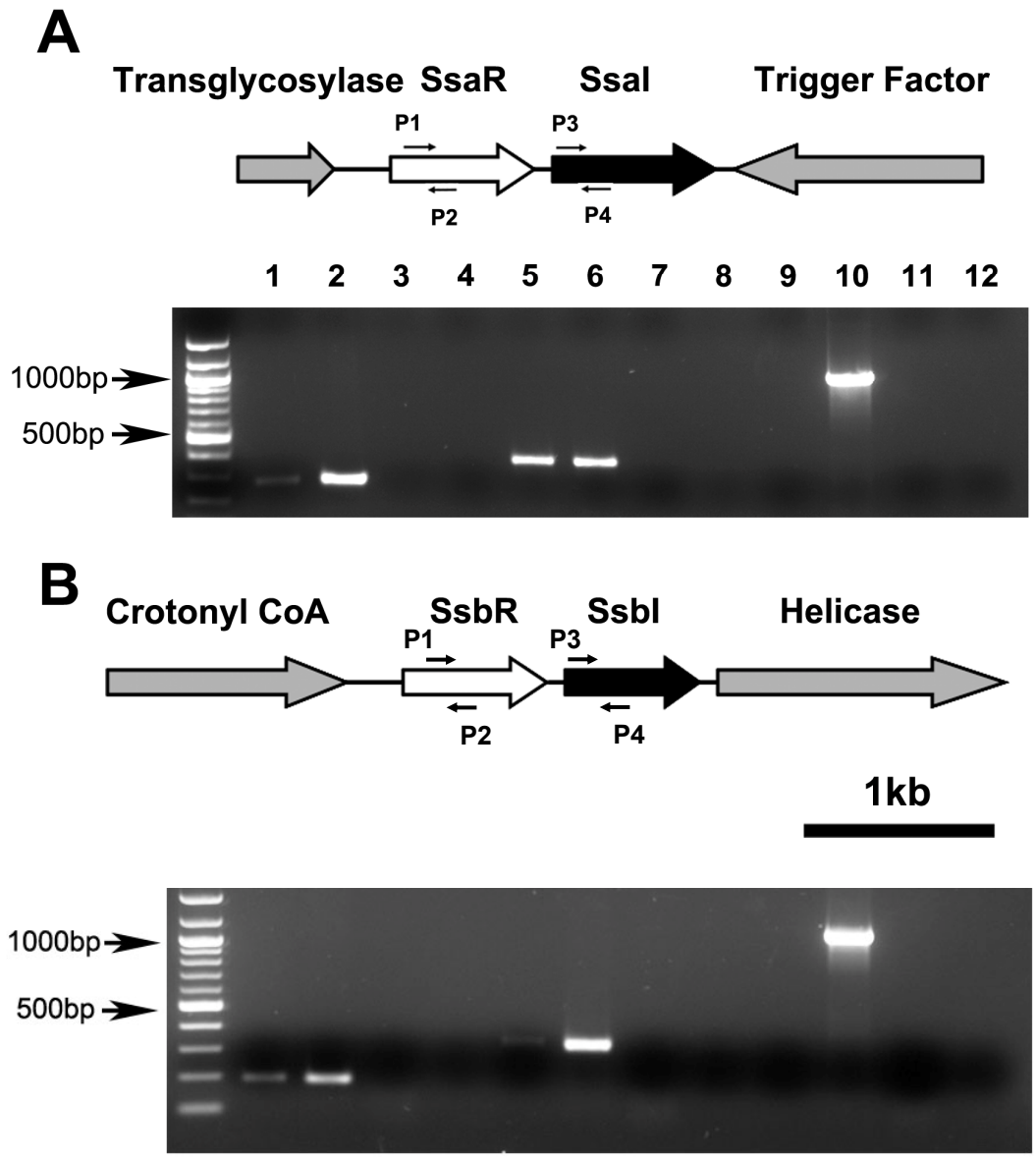


**Figure S2.** Gene maps of KLH11 *ssaR/ssaI* and *ssbR/ssbI* loci. Arrows represent genes.

A) SsaR (ZP_05123091) and SsaI (ZP_05123801) and are predicted to be 233 and 284 amino acids, respectively; Genbank accession numbers are ZP_05124568 for transglycosylase and ZP_05123238 for trigger factor. Primers used to test whether *ssaRI* are in the same operon are indicated as P1, P2, P3 and P4 (see Table S3, aP1, aP2, aP3, aP4). Lanes 1-4 were PCR results using primers P1 and P2, lanes 5-8 used primers P3 and P4, and lanes 9-12 used primers P1 and P4. Lanes 1, 5 and 9 used cDNA as template, lanes 2, 6 and 10 used genomic DNA as template, lanes 3, 7 and 10 used RNA as templates. Lanes 4, 8 and 12 were negative controls the primer sets.

B) SsbR (ZP_05123460) SsbI (ZP_05121795) are predicted to be 239 and 212 amino acids. ; Genbank accession numbers are ZP_05124465 for crotonyl CoA reductase and ZP_05122236 for helicase. Primers used to test whether *ssbRI* are in the same operon are indicated as P1, P2, P3 and P4 (see Table S3, primers bP1, bP2, bP3, bP4). The lanes are organized the same as in panel A.

## Zan et al., Supplementary Figure 2

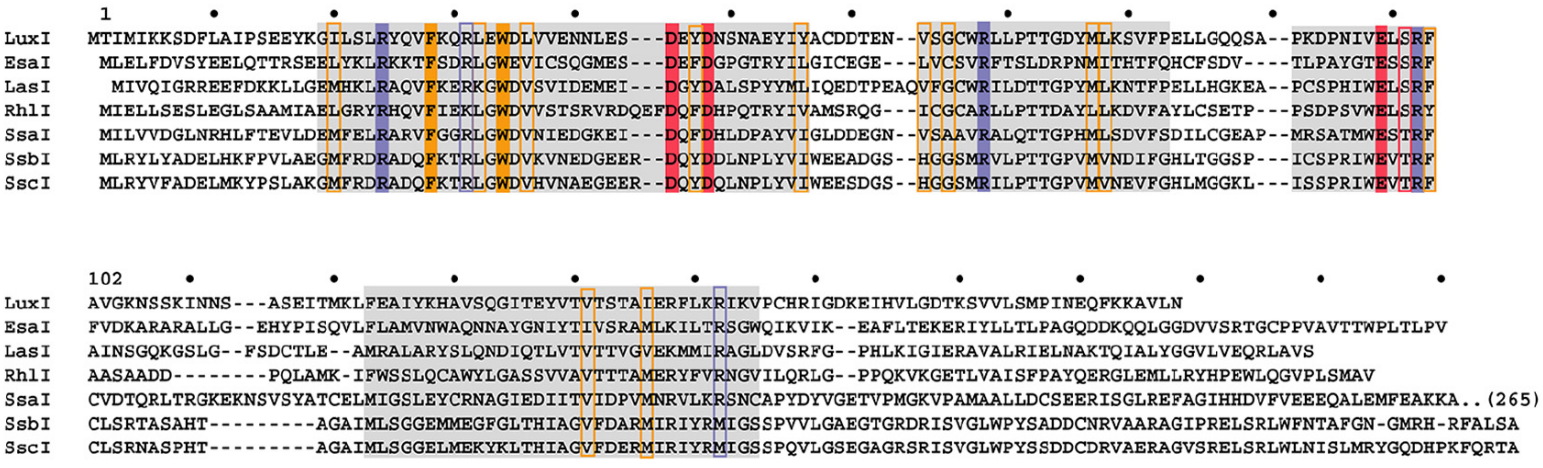


**Figure S3.** Sequence alignment of selected AHL synthases. The grey shaded regions are the most conserved sequence blocks within the AHL synthase family. Residues are colored red to indicate acidic or hydrophilic, blue for basic, and orange for other. Shaded residues are absolutely conserved and the boxed residues are the most similar regions within the family.

## Zan et al., Supplementary Figure 3

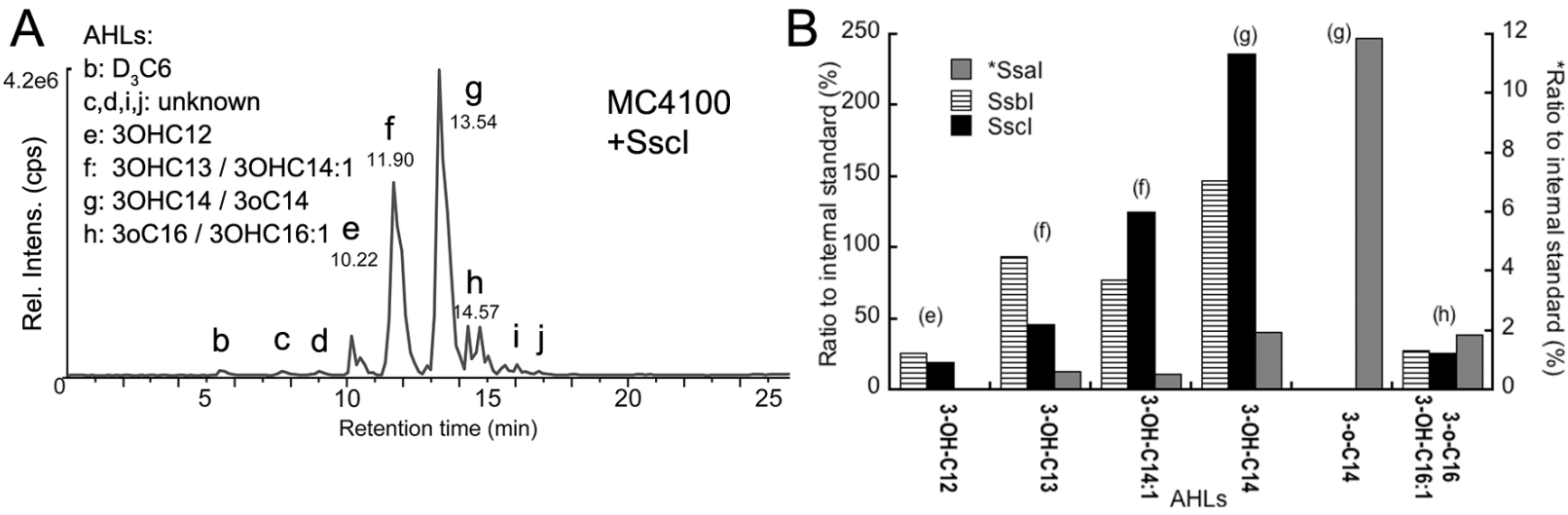


**Figure S4. Mass spectrometric analysis of plasmid-expressed SscI-directed AHLs from *E. coli*.** Cultures of *E. coli* MC4100 expressing plasmid-borne *sscI* were extracted and subjected to reverse-phase chromatographic separation prior to tandem MS analysis using the precursor ion-scanning mode (transitions were monitored for precursor [M + H]+ -> m/z 102) for (A) MC4100 + SscI. The peaks in the chromatograms are labeled with lettering and the AHLs are noted. (B) The relative amounts of the known AHLs in *E. coli* MC4100 derivatives harboring $P_{lac}$ expression plasmids with SsaI, SsbI, and SscI are shown in bar graphs, with the peak label above each set of bars. The plot shows the ratio of the area of the transition for each AHL to m/z 102 to the same transition for the internal standard D3-C6-HSL. The SsbI/SscI y axis is on the left, and the SsaI y axis is on the right.

## Zan et al., Supplementary Figure 4

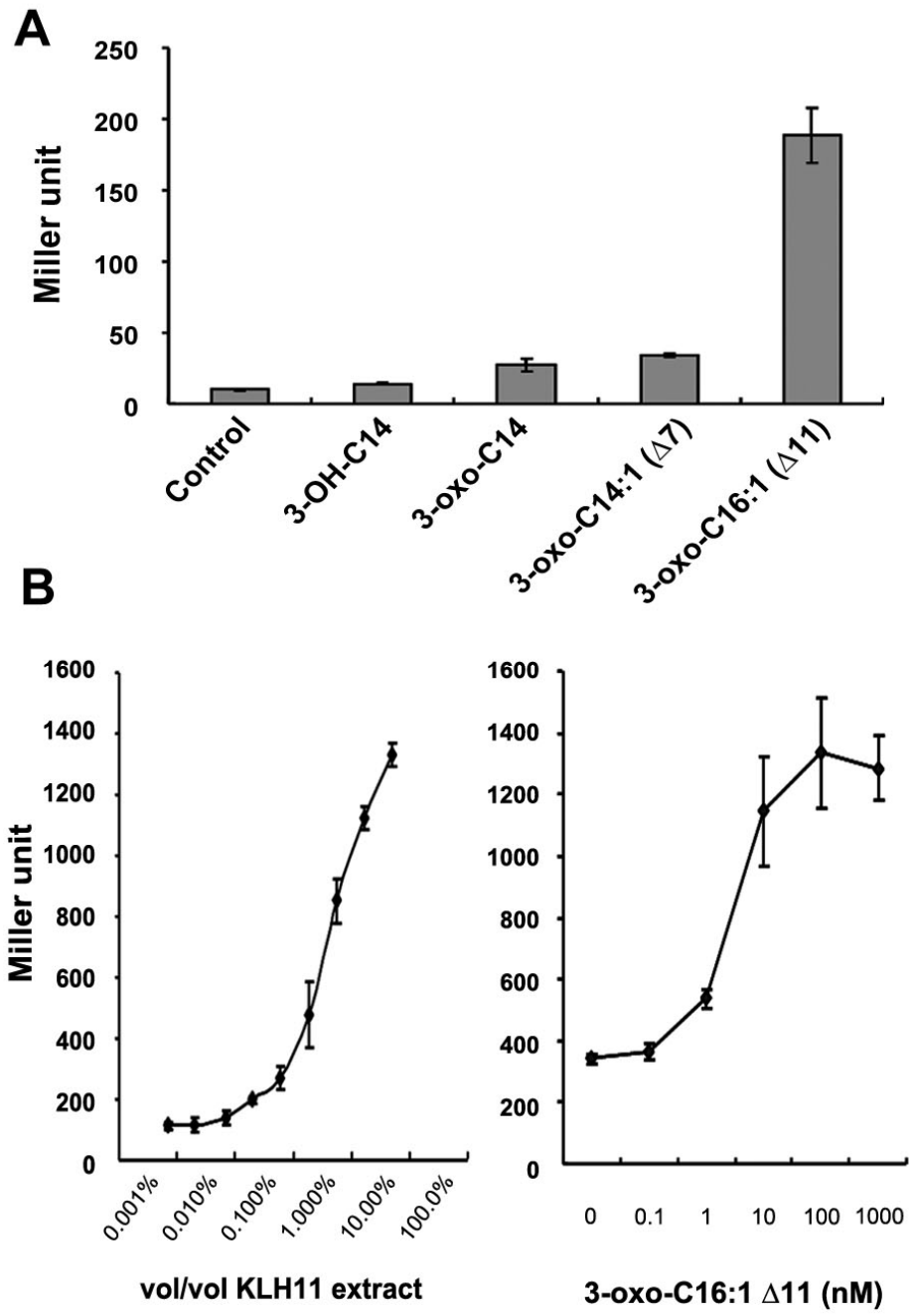


**Figure S5.** Activation of *ssaI* in response to synthetic AHLs (A) Activation of *ssaI-lacZ* fusion with synthetic long chain AHLs. KLH11 *ssaI-* carrying the integrated *ssaI-lacZ* fusion. The final concentration of 3-OH-C14 used was 20 μM and the other 3 AHLs were 2 μM (B) Dose responsive activation of *ssaI-lacZ* by SsaR ($P_{lac}$-*ssaR*) in *A. tumefaciens* NTL4 background. Different concentrations of the crude organic extract of KLH11 cultures (%, v/v) and 3-oxo-C16:1 Δ11 (nM) were added at the time of culture inoculation. Miller Units are averages of assays performed in triplicate and error bars are standard deviations.

## Zan et al., Supplementary Figure 5

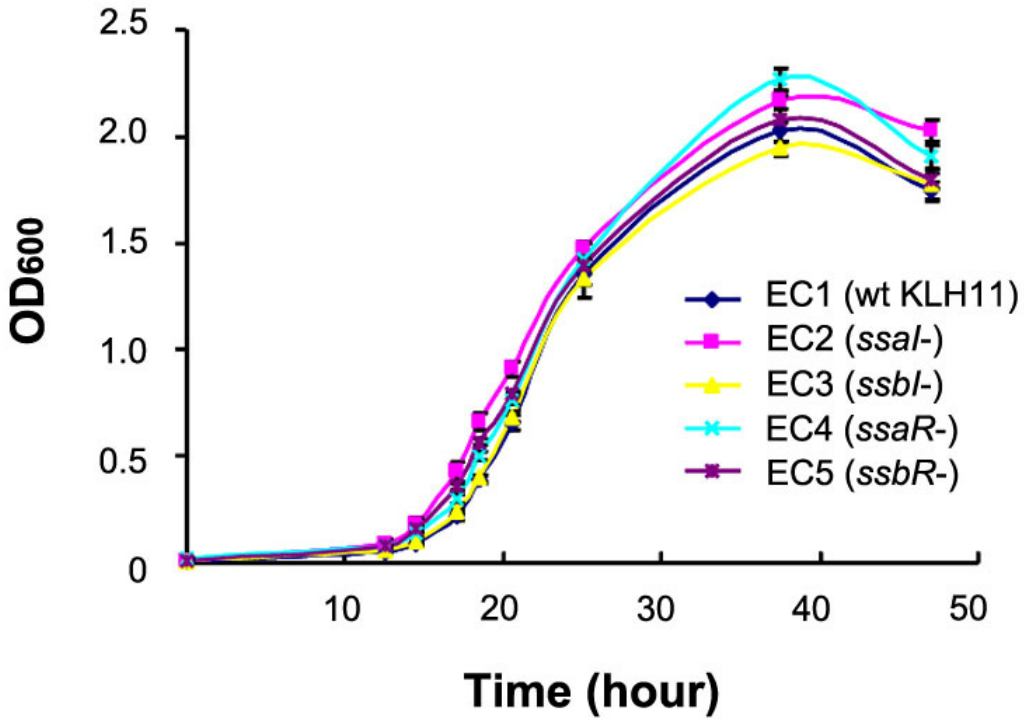


**Figure S6**. Growth curves of KLH11 and different QS mutants. Average $OD_{600}$ of 5 ml cultures in MB2216. Cultures were grown in triplicate.

## Zan et al., Supplementary Figure 6

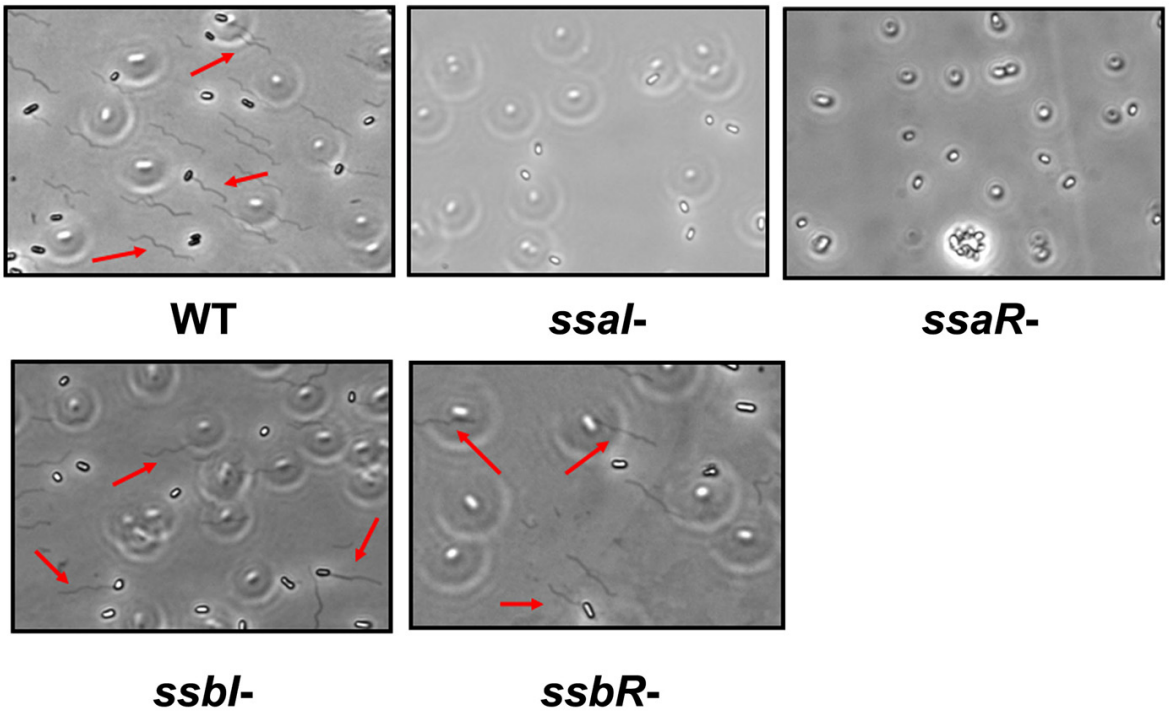


**Figure S7.** Flagellar staining of KLH11 quorum-sensing mutants. Stained cells from late stage cultures were viewed under phase contrast microscopy with 100X lens. Wild type (EC1), *ssaI*- (EC2), *ssbI*- (EC3), *ssaR*- (EC4), and *ssbR*- (EC5). Red arrows indicate stained flagella.

## Zan et al., Supplementary Figure 7