

Supporting Information for

Natural and synthetic inhibitors of a phage-encoded quorum-sensing receptor affect phage-host dynamics in mixed bacterial communities

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Ahyl and both C4-HSL and C8-HSL solubilize $LUXR_{ARM81d}$.

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Figure S1

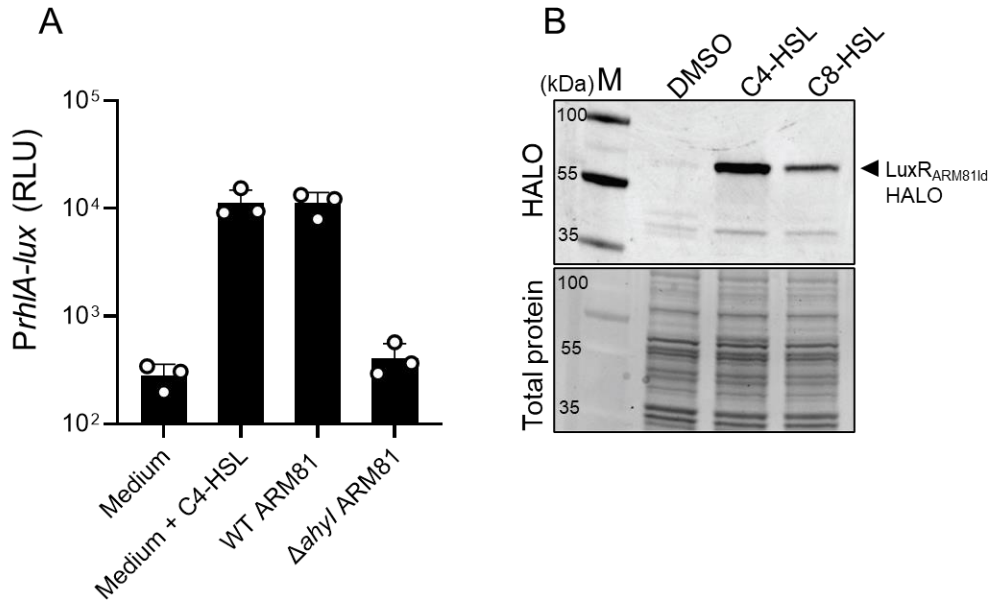


Figure S1. The ARM81 *ahyl* gene encodes the C4-HSL-producing synthase Ahyl and both C4-HSL and C8-HSL solubilize LuxR_{ARM81d}.

(A) *PrhIA-lux* expression from *E. coli* grown in medium supplemented with C4-HSL, cell-free culture fluids from WT *Aeromonas* sp. ARM81 (C4-HSL⁺), or cell-free culture fluids from Δ*ahyl* *Aeromonas* sp. ARM81 (C4-HSL⁻). The *E. coli* strain carries a plasmid with *PrhIA-lux* and a second plasmid with arabinose-inducible pBAD-*rhIR*. The *rhIA* promoter and *rhIR* gene both come from *P. aeruginosa*. In this system, RhIR only activates *PrhIA-lux* expression when C4-HSL is supplied exogenously. All media contained 0.1% arabinose.

(B) Western blot (top) and total protein (bottom) showing LuxR_{ARM81d}-HALO in the soluble fractions of *E. coli* supplied with 75 μM of the indicated HSL or an equivalent volume of DMSO. Detection of the HALO-reactive fluorophore requires that the HALO tag be properly folded. Thus, LuxR_{ARM81d}-HALO band intensity is a measure of folded LuxR_{ARM81d} in the soluble fraction of each sample. M denotes molecular weight marker (representative bands are labeled). kDa is kilodalton.

Table S1. Strains used in this study.

Strain	Genotype	Reference
<i>E. coli</i> TOP10	F- <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) ϕ 80 <i>lacZ</i> Δ M15 Δ <i>lacX74</i> <i>recA1</i> <i>araD139</i> Δ (<i>ara-leu</i>)7697 <i>galU</i> <i>galK</i> λ - <i>rpsL</i> (Str ^R) <i>endA1</i> <i>nupG</i>	Invitrogen
<i>E. coli</i> BL21	<i>fhuA2</i> [<i>lon</i>] <i>ompT</i> <i>gal</i> [<i>dcm</i>] Δ <i>hsdS</i>	Invitrogen
<i>Aeromonas</i> sp. ARM81 (JSS-3155)	WT	(Dziewit and Radlinska, 2016)
<i>Aeromonas</i> sp. ARM81 (JSS-3388)	Δ <i>ahyl</i>	This study
<i>Vibrio fischeri</i> ES114	WT	Bassler lab strain collection
	Δ <i>luxI</i>	Bassler lab strain collection
	Δ <i>ainS</i>	Bassler lab strain collection
	Δ <i>luxI</i> Δ <i>ainS</i>	Bassler lab strain collection

Table S2. Primers used in this study.

Name	Sequence (5 - 3)	Purpose; Template
JSO-2192	GTTTGCGGCCGCACGTTTTTACCCCC CCAAAGCC	<i>ahyI</i> upstream flanking DNA for insert into pFOG with NotI site; from <i>Aeromonas</i> sp. ARM81
JSO-2193	TTAACACTCTGTTATTACAAATGAGCA AAAAAAGTCCCGGGA	<i>ahyI</i> upstream flanking DNA for insert into pFOG; from <i>Aeromonas</i> sp. ARM81
JSO-2194	TTTGTAATAACAGAGTGTTAATT	<i>dhfr</i> cassette amplification; from EZ-DHFR Tn5
JSO-2195	TTAATTAAGCCTGGCACAGCGG	<i>dhfr</i> cassette amplification; from EZ-DHFR Tn5
JSO-2196	CGCTGTGCCAGGCTTAATTAACATTC ATGCCTCTCCATTCA	<i>ahyI</i> downstream flanking DNA for insert into pFOG; from <i>Aeromonas</i> sp. ARM81
JSO-2197	GTTTTGTTTAAACACATGGGGATGCG TTCCTGTGA	<i>ahyI</i> downstream flanking DNA for insert into pFOG with PmeI site; from <i>Aeromonas</i> sp. ARM81
JSO-1566	CATTAATTAACCTCCTGAATTCGGAA GCGAT	pT7 backbone amplification for Gibson assembly; pH6HTN plasmid
JSO-2035	TAATAGTCAACCGCTGAGCAATAACT AGC	pT7 backbone amplification for Gibson assembly; pH6HTN plasmid
JSO-2176	GCTTTGTATATGCCCCACCTA	qPCR; phage ARM81Id
JSO-2177	GCAATCAGACATCAGTGCCAAG	qPCR; phage ARM81Id
JSO-2106	CTTCTCTTCCGAGCGTTACGAC	qPCR; <i>Aeromonas</i> sp. ARM81 host (<i>rpoB</i>)
JSO-2107	GGTCGATATCGTCCACTTCGTC	qPCR; <i>Aeromonas</i> sp. ARM81 host (<i>rpoB</i>)

Table S3. Plasmids used in this study.

Plasmid name (informal)	Strain ID (formal)	Relevant fragment	Marker, Origin	Source
pTetA- <i>xre</i> _{ARM81d} - <i>luxR</i> _{ARM81d}	JSS-3237	<i>xre</i> _{ARM81d} - <i>luxR</i> _{ARM81d}	Amp, pBR322	(Silpe <i>et al.</i> , 2022)
pTetA- <i>xre</i> _{ARM81d} - <i>luxR</i> _{ARM81d}	JSS-3326	<i>xre</i> _{ARM81d} - <i>luxR</i> _{ARM81d}	Cm, p15A	(Silpe <i>et al.</i> , 2022)
PsmORF _{ARM81d} - <i>lux</i>	JSS-3235	PsmORF _{ARM81d} - <i>lux</i>	Kan, p15A	(Silpe <i>et al.</i> , 2022)
pFOG- Δ <i>ahyl::dhfr</i>	JSS-3387	Δ <i>ahyl::dhfr</i>	Gent, oriR6 κ	This study
pH6HTC-pT7- <i>luxR</i> _{ARM81d} -HALO-HIS	JSS-1868	<i>luxR</i> _{ARM81d} -HALO-HIS	Amp, pBR322	(Silpe and Bassler, 2019)