Supporting Information Appendix for:

A novel aryl-homoserine lactone quorum-sensing signal produced by a dimorphic prosthecate bacterium

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Supplemental Table S1. Uniprot and organism identifiers, synthesized signals and substrate preference for acyl-HSL synthases in Figure 1A

Uniprot id	Organism	Protein name	Acyl-HSL signal	CoA or ACP[a]
A0A0P6VH04_9RHIZ	*Prostechomicrobium hirschii* 16	HirI	PA-HSL	CoA
Q6NCZ6_RHOPA	*Rhodopseudomonas palustris* CGA009	RpaI	pC-HSL	CoA
A4YLT1_BRASO	*Bradyrhizobium* sp ORS278	CinI	Cinn-HSL	CoA
C5B506_METEA	*Methylobacterium extorquens* AM1	MsaI	C8-HSL	CoA
Q07P70_RHOPA	*Rhodopseudomonas* sp. BisA53		IV-HSL	CoA
BJAI_BRADU	*Bradyrhizobium japonicum* USDA 100	BjaI	IV-HSL	CoA
RHLI_PSEAE	*Pseudomonas aeruginosa* PAO1	RhlI	C4-HSL	ACP
A6V1U9_PSEEA7	*Pseudomonas aeruginosa* PA7	RhlI	C4-HSL	ACP
Q939D0_PSECL	*Pseudomonas chloroaphis (aerofaciens)*	CsaI	C4-HSL	ACP
PHZI_PSEFL	*Pseudomonas fluorescens*	PhzI	3OHC6-HSL	ACP
A0A0H3HXG1_BURPE	*Burkholderia pseudomallei* 1026b	BpmI1	C8-HSL	ACP
Q4VSJ8_BURGL	*Burkholderia glumae*	TofI	C8-HSL	ACP
A0A0H2WCK4_BMAI1	*Burkholderia mallei* ATCC 23344	BmaI1	C8-HSL	ACP
A0ZSH0_SHEH	*Shewanella (Alteromonas) hanedai*			ACP
LUXI_ALIFS	*Aliivibrio (Vibrio) fischeri*	LuxI	3oxoC6-HSL	ACP
A6CUQ4_9VIBR	*Vibrio shilonii* AK1			ACP
A0A0Q2RV31_VIBR	*Vibrio furnissii*			ACP
B2JQL0_PARP8	*Paraburkholderia phymatum*			ACP
D9SG23_GALCS	*Gallionella capsiferriformans* ES-2			ACP
LASI_PSEAE	*Pseudomonas aeruginosa* PAO1	LasI	3oxoC12-HSL	ACP
Q6ITU1_PSEFL	*Pseudomonas fluorescens*			ACP
E6VLG8_RHOPX	*Rhodopseudomonas palustris* DX-1			ACP
G7D671_BRAJP	*Bradyrhizobium japonicum USDA 6*			ACP
Q93UW5_RHIRD	*Rhizobium radiobacter*			ACP
F7XZ38_RHIFR	*Sinorhizobium fredii* GR64			ACP
TRAI_AGRFC	*Agrobacterium tumefaciens* C58	TraI	3oxoC8-HSL	ACP
H0HH08_RHIRD	*Agrobacterium tumefaciens* 5A			ACP
EAGI_ENTAG	*Enterobacter (Pantoea) agglomerans*	EagI	3oxoC6-HSL	ACP
Q7CGP3_YERPE	*Yersinia pestis*	YspI	3oxoC6-HSL	ACP
ESAI_PANSE	*Pantoea (Erwinia) stewartii*	EsaI	3oxoC6-HSL	ACP
YENI_YEREN	*Yersinia enterocolitica*	YenI	C6-HSL	ACP
SWRI_SERLI	*Serratia liquefaciens*	SwrI	C4-HSL	ACP
CARI_PECCC	*Pectobacterium (Erwinia) carotovorum*	CarI	3oxoC6-HSL	ACP
ECHI_DICCH	*Dickeya (Erwinia) chrysanthemi*	EchI	3oxoC6-HSL	ACP

[a]Known or predicted acyl substrate

Supplemental Table S2. Bacterial strains and plasmids

Strain, plasmid, or primer sequence	Relevant characteristic(s)	Source
Strains		
P. hirschii 16	Wild-type isolated from freshwater (ATCC 27832T)	(1, 2)
P. putida		
F1	Wild-type isolated from polluted creek	(3)
RLF1	Δ*paaF* phenylacetyl-CoA ligase (locus tag Pput_2480)	(4)
E. coli		
DH5α	*fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	NEB
S17-1	*thi, pro, hdsR, hdsM+ recA,* RP4-2 (Tc::Mu Km::Tn7)(4)	(5)
Plasmids		
$pBBP_{gdh}$	Broad-host vector with the *Rhodopseudomonas palustris gdh* (RPA0944) constitutive promoter; Gm^R	(6)
pUC18T-mini-Tn7T Gm-dsRedExpress	Source of *mCherry* gene template; Gm^R	(7)
pLL1	$pBBP_{gdh}$ expressing *hirR* gene plus a P_{hirI}-*mCherry* fusion; PA-HSL bioassay reporter; Gm^R	This work
pLL2	$pBBP_{gdh}$ expressing a P_{hirI}-*mCherry* fusion (no *hirR*); Gm^R	This work
pHirI	$pBBP_{gdh}$ expressing *hirI*; Gm^R	This work
pMal-t-aiiA	AiiA lactonase fused to a maltose-binding protein (MalE)	(8)

Supplemental Table S3. Primers

pBBR-hirR-F	5'CATATTGTTCCGCATTCATCTGCACCCTCCTCCTCGCTGCAGATGATTTTCAGTTC	vector primer (forward)
pBBR-mcherry-R	5'CGGCGGCATGGACGAGCTGTACAAGTAGGCTGGCGTAATAGCGAAGAGGC	vector primer (reverse)
hirR-R	5'ACTGAAAATCATCTGCAGCGAGGAGGAGGGTGCAGATGAATGCGGAACAATATGCG	*hirR* primer (downstream)
hirR-F	5'CGCCCTTGCTCACCATCTGCACCCTCCTGAGCACCTTCATCTGCACCCTC	*hirR* primer (upstream)
mCherry-R	5'GTGCAGATGAAGGTGCTCAGGAGGGTGCAGATGGTGAGCAAGGGCGAGGA	*mCherry* primer (downstream)
mCherry-F	5'GCGGGCCTCTTCGCTATTACGCCAGCCTACTTGTACAGCTCGTCCATGCC	*mCherry* primer (upstream)
pBBR-hirI-F	5'TGAGCACCTTCATCTGCACCCTCCTCCTCGCTGCAGATGATTTTCAGTTC	For linear pBBPgdh forward primer
pBBR-hirI-R	5'ACAATTTCGCGCTTCTCGGCGCGGTCTGAGAGGCAGACAAGGTATAGGGC	For linear pBBPgdh reverse primer
pBBR-mCherry2-F	5'CAGGCATATCCCGTAGCCCTCAGGGGTTGCCTGCACCCTCCTCCTCGCTG	For linear pLL2 (no *hirR*) forward primer
pBBR-mCherry2-R	5'AAATCATCTGCAGCGAGGAGGAGGGTGCAGGCAACCCCTGAGGGCTACGG	For linear pLL2 (no *hirR*) reverse primer
hirI-F	5'CCGCCTCGCCGCCCTATACCTTGTCTGCCTCTCAGACCGCGCCGAGAAG	*hirI* gene upstream primer
hirI-R	5'CATAGAACTGAAAATCATCTGCAGCGAGGAGGAGGGTGCAGATGAAGGTG	*hirI* gene downstream primer

SUPPLEMENTAL LITERATURE CITED

1. Daniel, J. J., Givan, S. A., Brun, Y. V. & Brown, P. J. (2015) Draft genome sequence of *Prosthecomicrobium hirschii* ATCC 27832T. *Genome Announc* 19: 3.
2. Staley, J. T. (1984) *Prosthecomicrobium hirschii*, a new species in a redefined genus. *Intl J Syst Bacteriol* 34: 304-308.
3. Gibson, D. T., Hensley, M., Yoshioka, H. & Mabry, T. J. (1970) Formation of (+)-*cis*-2,3-dihydroxy-1-methylcyclohexa-4,6-diene from toluene by *Pseudomonas putida*. *Biochem* 9: 1626-1630.
4. Luu, R. A., Schneider, B. J., Ho, C. C., Nesteryuk, V., Ngwesse, S. E., Liu, X., Parales, J. V., Ditty, J. L. & Parales, R. E. (2013) Taxis of *Pseudomonas putida* F1 toward phenylacetic acid is mediated by the energy taxis receptor Aer2. *Appl Environ Microbiol* 79: 2416-2423.
5. Simon, R., Priefer, U. & Puhler, A. (1983) A broad host range mobilitization system for in vivo genetic engineering: transposon mutagenesis in Gram-negative bacteria. *Nature Biotech* 1: 784-791.
6. McKinlay, J. B. & Harwood, C. S. (2010) Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. *Proc Natl Acad Sci USA* 107: 11669-11675.
7. Choi, K. H. & Schweizer, H. P. (2006) mini-Tn7 insertion in bacteria with single attTn7 sites: example *Pseudomonas aeruginosa*. *Nat Protoc* 1: 153-161.
8. Thomas, P. W. & Fast, W. (2011) Heterologous overexpression, purification, and in vitro characterization of AHL lactonases. *Meth Mol Biol* 692: 275-290.