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Dialkylresorcinols as bacterial signaling molecules

Supplementary Information Appendix

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SUPPLEMENTARY NOTE

Bacterial strains and plasmids

All strains used in this study are listed in SI Appendix, Table S1, the plasmids in SI Appendix, Table S2, and oligonucleotides in SI Appendix Table S3. *E. coli* strains were grown aerobically at 37°C in LB medium [10% (w/v) peptone, 5% (w/v) yeast extract, 10% (w/v) NaCl] or M63 minimal medium (1), whereas *P. asymbiotica* strains were grown aerobically at 37°C in Casein-Soya-Peptone (CASO) medium [10% (w/v) peptone of casein, 5% (w/v) peptone of soy flour, 5% (w/v) NaCl]. For preparation of solid media, 1.5% (w/v) agar was added. CASO agar was supplemented with 0.1% (w/v) pyruvate. Antibiotics were added for selection in the following concentration: ampicillin (100 µg ml^{-1}), carbenicillin (100 µg ml^{-1}), kanamycin (50 µg ml^{-1}), chloramphenicol (17 µg ml^{-1}), gentamicin (20 µg ml^{-1}), and streptomycin (20 µg ml^{-1}). Media were supplemented with 50 µg ml^{-1} aminolevulinic acid for growth of *E. coli* ST18. For production of DARs strain *Photorhabdus asymbiotica* PB68.1 was used. *E. coli* ST18 (SI Appendix, Table S1) was used as donor strain for conjugation of plasmid pMRS3-1-*pauR*-AB (this study) into *P. asymbiotica* PB68.1. Plasmid pUC19-Kan (Dr. Heinrich Jung, LMU München, lab collection) was used for cloning the 600 bp homology arms of the *P. asymbiotica pauR* gene, up- and downstream of a kanamycin resistance cassette to generate a *pauR* knockout in *P. asymbiotica*. For transfer into *P. asymbiotica*, this kanamycin-cassette surrounded by the *pauR*-homology arms was later brought into conjugation suicide plasmid pMRS101 (2) resulting in plasmid pMRS3-1-*pauR*-AB (see detailed description below). Plasmid pBAD-Cherry/*pcfA* (this study) was used as reporter plasmid to analyze P_{pcfA} activity.

Construction of plasmids

For construction of the conjugation plasmid pMRS3-1-*pauR*-AB, a kanamycin cassette flanked by 600 bp homology arms representing the *pauR* up- (A-site) and downstream area (B-site) was generated. The homology arms were amplified by PCR with Phusion© polymerase (New England Biolabs, Frankfurt) using genomic DNA of *P. asymbiotica* as template and primers PauR_FA_SacI_fwd and PauR_FA_XmaI_rev, and PauR_FB_SalI_fwd and PauR_FB_HindIII_rev, respectively. The A-site was then cloned upstream of the kanamycin cassette in

plasmid pUC19-Kan using restriction sites SacI and XmaI resulting in plasmid pUC-Kan-*pauR*-FA, then the B-site was cloned into plasmid pUC-Kan-*pauR*-FA using restriction sites SalI and HindIII resulting in plasmid pUC-Kan-*pauR*-AB. Then, the complete kanamycin cassette flanked with A- and B-site was amplified by PCR with primers pUC-ApaI_sense and pUC-SpeI_anti, cut with ApaI and SpeI, and the 2.7 kbp DNA fragment was ligated with equally treated vector pMRS101 resulting in plasmids pMRS3-*pauR*-AB. As last step, the pMB1 origin was removed from plasmid pMRS3-*pauR*-AB by treatment with restriction enzyme NotI and re-ligating the 9.5 kbp vector backbone resulted in suicide plasmid pMRS3-1-*pauR*-AB that was finally transferred into *E. coli* strain ST18. For construction of pBAD-Cherry, *mCherry* (750 bp) was amplified by PCR using primers Cherry_AflII_fwd and Cherry_SacI_rev using the Plasmid pBR-Cherry (3) as template, and cloned into pBAD33 (4) using restriction sites AflII and SacI. Expression *mCherry* is not under the control of the arabinose inducible *araBAD* promoter (4). Correct construction was verified by sequence analysis using primer mCherry-Seq_fwd.

For construction of pBAD-Cherry/*pcfA*, 250 bp of the region upstream the *pcfABCDEF* operon was amplified by PCR using primers P04068_XmaI_fwd and P04068_BamHI_rev using *P. asymbiotica* genomic DNA as template, and cloned into plasmid pBAD33-Cherry (this study) using restriction sites XmaI and XbaI. Correct insertion was verified by sequence analysis using primer mCherry-Seq_fwd. For construction of pBAD-Cherry/*pauR*, 400 bp of the region upstream *pauR* was amplified by PCR using primers P04062_BamHI_fwd and P04062_XmaI_rev using *P. asymbiotica* genomic DNA as template, and cloned into plasmid pBAD33-Cherry (this study) using restriction sites BamHI and XmaI. Correct insertion was verified by sequence analysis using primer mCherry-Seq_fwd. For construction of pBRCherry/*darA*, 390 bp of the region upstream of the *darABC* operon (*pau02400-pau02402*) was amplified by PCR using primers PdarA_XmaI_fwd and PdarA_BamHI_rev using *P. asymbiotica* genomic DNA as template, and cloned into plasmid pBAD33-Cherry (this study) using restriction sites XmaI and BamHI. Correct insertion was verified by sequence analysis using mCherry-Seq_fwd.

The complete *pcfABCDEF/pauR* genomic region was cloned into expression vector pBAD24 so that the region is under control of the arabinose inducible *araBAD* promoter (4). The region was previously amplified in two parts by PCR using primers

Pau_4062_NheI_sense1 and Pau_4064_XmaI_anti1 or Pau_4064_XmaI_sense1 and Pau_4068_PstI_anti1, respectively, using genomic *P. asymbiotica* PB68.1 DNA as template. The two PCR products 3.7 kbp (*pauR/pcfEF*) and 5.1 kbp (*pcfABCD*) were cloned in two steps into pBAD24 using restriction sites NheI, XbaI and PstI resulting in plasmid pBAD/*pcfABCDEF/pauR*. For construction of the pBAD/*pcfABCDEF* plasmid, the region was previously amplified in two parts by PCR using primers Pau_4063_PstI_sense1 and Pau_4064_XmaI_anti1 or Pau_4064_XmaI_sense1 and Pau_4068_NheI_anti1, respectively, using genomic *P. asymbiotica* DNA as template and the two PCR products 2.6 kbp (*pcfEF*) and 5.1 kbp (*pcfABCD*) were cloned in two steps into pBAD24 using restriction sites PstI, XmaI and NheI.

For construction of pBAD24-His-*pauR*, *pauR* (717 bp) was amplified by PCR using the primers PAU4062-His-NheI_fwd and 4062_SalI_rev using *P. asymbiotica* PB68.1 genomic DNA as template, and cloned into plasmid pBAD24 (4) using restriction sites NheI and SalI. Correct insertion was verified by sequence analysis using primer pBAD24_Seq_sense. The generation of *pauR* variants was achieved with two-step PCR using the appropriate primer pairs and *P. asymbiotica* PB68.1 genomic DNA as template to gain the respective amino acid substitution (Pau_T62A_fwd and Pau_T62A_rev, Pau_Y66A_fwd and Pau_Y66A_rev or Pau_D75A_fwd and Pau_D75A_rev, respectively). The overlap PCR was performed using the primers PAU4062-His-NheI_fwd and 4062_SalI_rev and the fragment was cloned into plasmid pBAD24 using restriction sites NheI and SalI. Correct insertion was verified by sequence analysis using primer pBAD24_Seq_sense.

For construction of the reporter plasmid pBBR1-MCS5-TT-RBS-P_{pcfA}-*lux*, 250 bp of the region upstream of *pcfA* (*pau_04068*) was amplified by PCR using primers P04068_XmaI_fwd and P04068_XbaI_rev using *P. asymbiotica* PB68.1 genomic DNA as template, and cloned into plasmid pBBR1-MCS5-TT-RBS-*lux* (5) using restriction sites XbaI and XmaI. Correct insertion was verified by sequence analysis using primer pNTPS_Seq_fwd.

Competent cells and transformations

E. coli cells were made chemically competent using a modified RbCl method and transformed as described earlier (6). *P. asymbiotica* was made electrocompetent and transformed by electroporation. Cells of *P. asymbiotica* were cultivated aerobically in

CASO medium at 37°C up to an OD600 of 0.8-1.0. Then, cells were harvested by centrifugation at room temperature, the cell pellet was resuspended in the same volume of 10% (v/v) glycerol, and collected again by centrifugation. Cells were then washed in 1/2 of starting volume, and then in 1/20 of starting volume of 10% (v/v) glycerol, and then resuspended in 1/300 of starting volume in 10% (v/v) glycerol. For the following electroporation step, 60 µl of cell suspension were mixed with 100 ng plasmid-DNA, incubated at room temperature for 10 min, and then transferred into 0.2 cm electroporation cuvettes. Electroporation was performed with a pulse of 2.500 V for 4-6 msec. Subsequently, cells were removed from the cuvettes by flushing with 1 ml CASO medium, and incubated aerobically at 37°C for 1.5 h. The complete transformation samples were spread on appropriate agar plates and incubated at 37°C for two days.

Generation of the gene knockouts in *P. asymbiotica*

Generation of *P. asymbiotica* PB68.1 Δ*pauR* (*pau_04062*) was performed by homologous recombination of a kanamycin cassette replacing the *pauR* gene. The conjugative plasmid transfer from donor strain *E. coli* ST18 pMRS3-1-*pauR*-AB into *P. asymbiotica* PB68.1 was performed with the filter mating method (7). Therefore, the donor strain was cultivated in LB broth and the recipient strain *P. asymbiotica* PB68 in CASO medium up to an optical density (OD_{600}) of 0.6 (donor) or 0.8 (recipient). Then, a volume of 1 ml donor cells were harvested by centrifugation, washed three-times with CASO medium and resuspended in a volume of 30 µl. A volume of 5 ml of the recipient was harvested by centrifugation and resuspended in a volume of 30 µl. Donor and recipient were pooled and dropped onto a nitrocellulose filter that was positioned in the middle of a CASO agar plate. After incubation for 24 h at 37°C, the complete cell material was scraped from the filter, suspended in 1 ml CASO medium, spread onto CASO medium plates containing kanamycin and incubated for 2 days at 37°C. As the donor has a 5-aminolevulinic acid (ala) auxotrophy, this strain is not able to grow on CASO medium. Then, exconjugants were inoculated for 24 h in liquid CASO medium containing 10% (w/v) sucrose and kanamycin and then streaked on CASO medium containing 10% (w/v) sucrose and kanamycin. Clones were checked on streptomycin sensitivity by streaking on CASO agar containing streptomycin. Genomic DNA was prepared using the "Ultra-Clean Microbial DNA Isolation Kit" (Mo-Bio Laboratories Inc., Carlsbad, CA) of Kan^R,

Suc^R and Strep^S clones, and the correct replacement of the *pauR* gene with the kanamycin cassette was verified by PCR using primers annealing outside the 600 bp homology arms (PauR_check_fwd and Kan_LB_anti or PauR_check_rev and Kan_RB_sense, respectively) and DNA sequencing.

The *darB (pau_02401)* insertion mutant was generated using the suicide vector pNPTS138-R6KT (8). Briefly, a 577 bp fragment within *darB* was amplified with restriction site modified primers darB_KI_EcoRV_fwd and darB_KI_NheI_rev and cloned into pNPTS138-R6KT via NheI and EcoRV. The final construct, pNPTS138-R6KT-*darB,* was then transferred into *E. coli* ST18 by transformation, and subsequently used for conjugation with *P. asymbiotica* PB68.1. Mutants were verified genotypically by PCR using the primer DarAB_Check_rev1 and pDS132 vector specific primers pDS_Seq_fwd resulting in 2.9 kbp DNA fragments and DarAB_Check_fwd1 and pDS132 vector specific primers pDS_Seq_rev resulting in 3.2 kbp DNA fragments. The correct insertion mutant was further verified phenotypically by HPLC-MS analysis for the loss of DAR production.

Heatmap

The phylogenetic analysis was based on a 646 bp region of *recA* for different *Photorhabdus* and *Xenorhabdus* strains with *E. coli* as outgroup (9). The data obtained from the LC-MS spectra of the different strains was analyzed regarding the relative amount of produced compounds and connected to the results of the phylogenetic analysis. ESI HPLC MS analysis was performed with a Dionex UltiMate 3000 system coupled to a Bruker AmaZon X mass spectrometer using an Acquity UPLC BEH C18 1.7 µm RP column (Waters) as described previously (10).

Phylogenetic analysis

The phylogenetic analysis of ketosynthases and LuxR-like proteins was calculated using the PHYML (11) algorithm with standard parameters. DarA (WP_012794415.1), DarB (WP_012794414.1) and DarC (WP_012794409.1) from *Chitinophaga pinensis* DSM 2588, LuxI (WP_005423459.1), full-length LuxR (WP_005423460) and its HTH domain (aa 185-241) from *Aliivibrio fischeri* was used to search the *darB* encoding genomes. The underlying multiple sequence alignment was generated using the ClustalW (12) alignment also with standard parameters. For visualization and calculation of the alignment as well as the PHYML tree the

Geneious software (Biomatters Ltd., New-Zeeland) was used.

Table S1: Strains.

Strain	Genotype	Reference
***P. asymbiotica* PB68.1**	Wild-type isolate	(9)
***P. asymbiotica* ATCC 43949**	Wild-type isolate	(13)
P. asymbiotica* PB68.1 Δ*pauR	PB68.1 Δ*pauR::Km*^{R} (*pau_04062*)	This study
P. asymbiotica* PB68.1 *darB	PB68.1 *darB::Km*^{R} (*pau_02401*)	This study
***E. coli* LMG194**	F$^-$ Δ*lacX74 galE galK thi rpsL ΔphoA* (PvuII) Δ*ara714 leu*::Tn*10*	(4)
E. coli* S17-λ*pir	Tp^R Sm^R *recA thi pro hsdR*-M$^+$RP4: 2-Tc:Mu: Km Tn7 λ*pir*	Biomedal S.L., Seliva, Spain
***E. coli* ST18**	*E. coli* S17 λ*pir* Δ*hemA*	(14)
***E. coli* JM109**	*recA1 endA1 gyrA96 thi hsdR17 supE44*λ*relA1* Δ*(lac-proAB)*/F' *traD36 proA*$^+$*B*$^+$ *lacI*^{q}*lacZ*ΔM15	(15)
***E. coli* BL21 DE3**	F$^-$ *ompT gal dcm lon hsdSB*(*rB- mB*) λ(DE3)	(16)

Table S2: Plasmids.

Plasmid	Characteristics	Reference
pBAD24	Expression vector, arabinose inducible promoter, Amp^R	(4)
pBAD-*pcfABCDEF*	*pcfABCDEF* (*pau_04068-pau_04063*) operon in pBAD24	This study
pBAD-*pcfABCDEF*/*pauR*	*pcfABCDEF* operon and *pauR* (*pau_04068-pau_04062*) in pBAD24	This study
pBR-Cherry	*mcherry* in pBR322	(3)
pBAD33	Expression vector, arabinose inducible promoter, Cm^R	(4)
pBAD-Cherry	mCherry in pBAD33	This study
pBAD-Cherry/*pcfA*	*pcfA*-promoter upstream of *mcherry* in pBAD33	This study
pBAD-Cherry/*pauR*	*pauR*-promoter upstream of *mcherry* in pBAD33	This study
pBAD-Cherry/*darA*	*darA*-promoter upstream of *mcherry* in pBAD33	This study
pBAD24-*darABC/mtaA*	*darABC*-operon from *P. asymbiotica* and *mtaA*	(17)
pACYC-*bkdABC/ngrA*	*bkdABC*-operon and *ngrA* both from *P. luminescens*	(18)
pUC19	Cloning vector, Amp^R	(15)
pUC19-Kan	Km^R cassette in pUC19	(H. Jung, München, Lab Collection)
pUC-Kan-*pauR*-FA	600 bp upstream region of *pauR* cloned upstream of Km^R cassette	This study
pUC-Kan-*pauR*-AB	600 bp up- and downstream regions of *pauR* cloned up- and downstream of Kan^R (*pauR*-interposon)	This study
pMRS101	Conjugation vector, R6K ori, pMB1 ori, $Strep^R$, *sucB*	(2)
pMRS3-*pauR*-AB	*pauR*-interposon in pMRS101	This study
pMRS3-1-*pauR*-AB	*pauR*-interposon in pMRS101, ΔpMB1 ori	This study
pNPTS138-R6KT	$mobRP4^+$ *ori*-R6K *sacB*; suicide plasmid for deletions; Kan^R	(19)
pNPTS138-R6KT-*darB*	Intergenic region of 577 bp of *darB* in pNPTS138-R6KT	This study
pBAD24-His-*pauR*	*pauR* (*pau_04062*) in pBAD24 with N-	This study

	terminal His-tag	
pBAD24-His-*pauR*-T62A	Substitution of T62A in *pauR* (*pau_04062*) in pBAD24	This study
pBAD24-His-*pauR*-Y66A	Substitution of Y66A in *pauR* (*pau_04062*) in pBAD24	This study
pBAD24-His-*pauR*-D75A	Substitution of D75A in *pauR* (*pau_04062*) in pBAD24	This study
pBBR1-MCS5-TT-RBS-*lux*	*luxCDABE* and terminators lambda T0 rrnB1 T1 cloned into pBBR1-MCS5 for plasmid-based transcriptional fusions; Gm^R	(5)
pBBR1-MCS5-TT-RBS-P_{pcfA}-*lux*	*luxCDABE* under the control of the *pcfA* (*pau_04068*) promoter	This study

Table S3: Oligonucleotides.

Oligo	Sequence
PauR_FA_SacI_fwd	5′-TAGCCGAGCTCGCACCATCACCCTGTTTCAG-3′
PauR_FA_XmaI_rev	5′-TAGCCCCCGGGAAGATTTCTCTCATTAAATAAT-3′
PauR_FB_SalI_fwd	5′-TAGCCGTCGACTAATTAGAGCCCGATTAAAG-3′
PauR_FB_HindIII_rev	5′-TAGCCAAGCTTGGAAGACACGCTATTGCG-3′
P04068_XmaI_fwd	5′-TAGCCCCCGGGTTTTCGGTCAATGTGAAGAACAT-3′
P04068_BamHI_rev	5′-TAGCCGGATCCGAAATTTTATTTATATAGC-3′
P04062_XmaI_rev	5′-TAGCCCCCGGGAAGATTTCTCTCATTAAATAA-3′
P04062_BamHI_fwd	5′-TAGCCGGATCCCAACGCATCACATAACCCTG-3′
PdarA_XmaI_fwd	5′-TAGCCCCCGGGATGTTCTAACCTTTATGGGTA-3′
PdarA_BamHI_rev	5′-TAGCCGGATCCCAATTTTATTTATTATCTTG-3′
mCherry-Seq_fwd	5′-CCCTTAGTAACTTTTAGC-3′
PAU_4062_NheI_sense_1	5′TAGCCGCTAGCGGCACCGCTGGAGAACGACTTTCC-3′
PAU_4068_PstI_anti_1	5′TAGCCCTGCAGGGTCATTTATTTTATCCTATTCTATATG-3′
PAU_4064_XmaI_sense_1	5′TAGCCCCCGGGTATGCCTACTGGGATAGATTTTTATC-3′
PAU_4064_XmaI_anti_1	5′TAGCCCCCGGGGAAGTTAATTTGAGTGTTGCCCAGC-3′
PAU_4063_PstI_sense_1	5′TAGCCCTGCAGCTATGAAATATAATTCGCCAAAATACC-3′
PAU_4068_NheI_anti_1	5′TAGCCGCTAGCGGTCATTTATTTTATCCTATTCTATATG-3′
PAU4062-His-NheI_fwd	5′-GAGGAAGCTAGCCGCACCACCATCATCACCATCC CGGGATCTTATGAATACTTTATT-3′
4062_SalI_rev	5′-TAGCCGTCGACTTATATGATTAGATTATATGC-3′
pBAD24_Seq_sense	5′-GCCGTCACTGCGTCTTTTACTGG-3′
PAU_T62A_fwd	5′-TTTACACACAGAA GCA ATGGGTAA-3′
PAU_T62A_rev	5′-TTACCCAT TGC TTCTGTGTGTAAA-3′
PAU_Y66A_fwd	5′-CATGGGTAATGCTGATAAA-3′
PAU_Y66A_rev	5′-TTTATCAGCATTACCCATG-3′
PAU_D75A_fwd	5′-CATGACAGT GCT CAACTAATG-3′
PAU_D75A_rev	5′-CATTAGTTG AGC ACTGTCATG-3′
P04068_XbaI_rev	5′-TAGCC TCTAGA GAAATTTTATTTATATAGC-3′
pNTPS_Seq_fwd	5′-GTCATATTTGCCCTCCTGG-3′
PauR_check_fwd	5′-GTTAATGCTTCGATCCATCC-3′
PauR_check_rev	5′-GCAAATTCTCGGTGCATTCC-3′
Kan_RB_sense	5′-GGATTCATCGACTGTGGCCG-3′
Kan_LB_anti	5′-CAGTCATAGCCGAATAGCCT-3′
darB_KI_EcoRV_fwd	5′-TAGCCGATATCCCCAATAGATAATGATACAAT-3′
darB_KI_NheI_rev	5′-TAGCCGCTAGCCCGTGGTTTATTTTCAAGCA-3′
pDS_Seq_fwd	5′-GCATGGGCATAAAGTTGCC-3′
pDS_Seq_rev	5′-CTAAGCTCTCATGTTTGAAC-3′
DarAB_Check_fwd1	5′-GCCCGCTATGATAATAAAGGAGATAG-3′
DarAB_Check_rev1	5′-CCCGCTCATTTAAGTGTCTAAC-3′
Cherry_AflII_fwd	5′-TAGCCCTTAAGTTATTTGTATAGTTCATCC-3′
Cherry_SacI_rev	5′-TAGCC GAGCTC ATGGCAACTAGCGGCATGGT-3′

Table S4: The loss of PauR binding to compound 6 (DAR) *in silico* using the built-in residue scan function of MOE 2013.0802. A positive number indicates a loss of affinity or stability, respectively. To investigate the effect of single amino acid substitutions inside the PauR binding pocket considering the binding-affinity of PauR and it's ligand, a virtual mutagenesis was performed for T62, Y66 and D75. All three residues were replaced with alanine and with the in-built residue scan function of MOE 2013.0802 the ligand affinity and protein stability was predicted. For wild-type PauR an affinity and stability to the docked DAR (**6**) of -7.31 kcal/mol and -13.26 kcal/mol were calculated, respectively. The Y66A derivative showed the highest loss of ligand affinity with destabilizing increase of +1.64 kcal/mol for affinity and +4.27 kcal/mol for protein stability, for the D75A derivative a small change of ligand affinity (+0.39 kcal/mol) has been observed which could be explained with a rise in protein stability (-4.01 kcal/mol). The same is true for the T62A derivative, which showed a small loss of ligand affinity (+0.41 kcal/mol) while the protein stability is increased (-0.82 kcal/mol). These results indicate the change of DAR binding by these PauR derivatives and confirm the experimentally demonstrated importance for binding of Y66. As a control, similar experiments were performed with QscR (-10.68 kcal/mol affinity and -1.99 kcal/mol stability, respectively, for the QscR-AHL complex), which has also been characterized experimentally regarding amino acids required for AHL binding and all experimental data showing a decrease of AHL-binding could be confirmed *in silico*.

	Δ ligand affinity	**Δ protein stability**
PauR derivative		
T62A	+0.41	-0.82
Y66A	+1.64	+4.27
D75A	+0.39	-4.01
QscR derivative		
S38A	+0.10	-2.47
G40F	+2.94	+11.47
S56G	+0.60	+2.07
D75A	+2.64	-2.37
V78F	+2.08	+1.79
L82F	+0.11	+2.95

Table S5: **Ketosynthases (KS) used for the phylogenetic tree.** Referred to Fuchs *et al*. (17), all newly identified *darB* genes are shown in red.

	Protein	Organism	Accession number
	Closest BLAST-P hits for PpyS		
1	3-Oxoacyl-ACP synthase III	*Burkholderia* sp. CCGE1001	YP_004230959
2	3-Oxoacyl-ACP synthase III	*Burkholderia phenoliruptrix* BR3459a	YP_006793509
3	3-Oxoacyl-ACP synthase III	*Burkholderia* sp. CCGE1003	YP_003910175
4	3-Oxoacyl-ACP synthase III	*Burkholderia phytofirmans* PsJN	YP_001889944
5	hypothetical protein	*Chlorogloeopsis*	WP_016876568
6	hypothetical protein	*Anabaena* sp. PCC 7108	WP_016949109
7	*PpyS*	*P. luminescens subsp. laumondii*	AGO97060
8	3-Ketoacyl-CoA thiolase	*Pseudomonas* sp. GM30	WP_007967127
9	3-Oxoacyl-ACP synthase I	*Xenorhabdus bovienii* SS-2004	YP_003469508
10	3-Oxoacyl-ACP synthase	*Xenorhabdus nematophila* ATCC 19061	YP_003713506
11	3-Oxoacyl-ACP synthase	*Xenorhabdus nematophila* ATCC 19061	WP_010847197
	Closest BLAST-P hits for XclC		
12	3-Oxoacyl-ACP synthase	*C. acetobutylicum*	NP_347450.1
13	3-Oxoacyl-ACP synthase	*P. lactis*	WP_007130623.1
14	3-Oxoacyl-ACP synthase	*B. thuringiensis*	YP_006930640.1
15	3-Oxoacyl-ACP synthase	*B. sp. 1NLA3E*	YP_007911827.1
16	3-Oxoacyl-ACP synthase	*O. scapharcae*	WP_010098042.1
17	3-Oxoacyl-ACP synthase	*P. polymyxa*	YP_003947618.1
18	3-Oxoacyl-ACP synthase	*P. polymyxa*	YP_003871436.1
19	3-Oxoacyl-ACP synthase	*P. sp. Aloe-11*	WP_007431139.1
20	3-Oxoacyl-ACP synthase	*P. terrae*	YP_005077926.1
21	3-Oxoacyl-ACP synthase	*P. peoriae*	WP_010345468.1
	FabH		
22	CorB	*Corallococcus coralloides*	ADI59524
23	Myxopyronin ketosynthase	*Myxococcus fulvus*	AGS77282
24	FabHB	*B. subtilis*	NP_388898
25	FabH	*N. punctiforme*	YP_001865657
26	3-oxoacyl-ACP synthase	*B. subtilis*	NP_389015.1
27	FabH	*A. fabrum*	NP_354198
28	FabH	*P. luminescens*	NP_930069
29	FabH	*E. coli*	NP_287225
30	FabH	*S. griseus*	YP_001826619
31	FabH	*S. echinatus*	AAV84077
32	NP_626634	*S. coelicolor A3(2)*	NP_626634
33	FabH	*S. avermitilis*	BAC73499
34	Q54206	*S. glaucescens*	Q54206
35	FdmS	*S. griseus*	AAQ08929
36	CAM58805_S._sp._BenQ	*S. sp. A2991200*	CAM58805
37	ZhuH 1MZJ	*S. sp. R1128*	AAG30195
38	FrnI	*S. roseofulvus*	AAC18104
39	AlnI	*S.* sp CM020	ACI88883
40	OleA	*Xanthomonas campestris* pv. *campestris*	3S21 (PDB)
	KS type III PKS		
41	Chs-like	*R. baltica*	NP_868579
42	BPS (PLN03172)	*H. androsaemum*	Q8SAS8
43	CHS H. (PLN03173)	*H. androsaemum*	Q9FUB7

44	CHS9	*M. sativa*	AAA02827
45	STS	*P. quinquefolia*	AAM21773
46	BAS	*R. palmatum*	AAK82824
47	BpsA	*Bacillus subtilis str. 168*	NP_390087
48	MXAN_6639	*M. xanthus*	YP_634756
49	PKS10	*M. tuberculosis*	NP_216176
50	PKS11	*M. tuberculosis*	NP_216181
51	Cpz6 Capramyzin ketosynthase	*Streptomyces* sp. MK730–62F2	(20)
52	Germicidin synthase	*Streptomyces coelicolor*	3V7I_A
53	RppA S	*S. antibioticus*	BAB91443
54	RppA	*S. avermitilis*	NP_828307
55	RppB	*S. antibioticus*	BAB91444
	KS adjacent to XclA homologues		
56	3-Oxoacyl-ACP synthase	*C.* sp. PCC 7822	YP_003899922.1
57	3-Oxoacyl-ACP synthase	*N. punctiforme*	YP_001865657.1
58	3-Oxoacyl-ACP synthase	*A. cylindrica*	YP_007155727.1
	Closest BLAST-P hits for XclB		
59	3-Oxoacyl-ACP synthase III	*B.* sp. EniD312	WP_009111263.1
60	3-Oxoacyl-ACP synthase III	*A. nasoniae*	CBA73264.1
61	3-Oxoacyl-ACP synthase III	*P. carotovorum*	WP_010301235.1
62	3-Oxoacyl-ACP synthase III	*P. pacifica*	WP_006975318.1
63	3-Oxoacyl-ACP synthase III	*C. stagnale*	YP_007317906.1
64	3-Oxoacyl-ACP synthase III	*N. punctiforme*	YP_001865628.1
65	3-Oxoacyl-ACP synthase III	*R.* sp. PCC 7116	YP_007056099
66	3-Oxoacyl-ACP synthase III	*S. cyanosphaera*	YP_007130807.1
67	3-Oxoacyl-ACP synthase III	*Calothrix* sp. PCC 6303	YP_007138278
68	3-Oxoacyl-ACP synthase III	*N. punctiforme*	YP_001868566.1
69	3-Oxoacyl-ACP synthase III	*R.* sp. PCC 7116	YP_007057764.1
	ChlB6; CerJ; KSIII DpsC-like		
70	ChlB6	*S. antibioticus*	AAZ77679
71	CerJ	*S. tendae*	AEI91069
72	CosE	*S. olindensis*	ABC00733
73	DpsC	*S. peucetius*	AAA65208
74	AknE2	*S. sp. SPB74*	ZP_04991255.1
75	AknE2	*S. galilaeus*	AAF70109
76	BAB72048	*S. galilaeus*	BAB72048
77	PokM2	*S. diastatochromogenes*	ACN64832
78	CalO4	*S. aurantiaca*	ZP_01462124
79	FabH	*S. erythraea*	YP_001107471
80	NdasDRAFT_3133	*N. dassonvillei*	ZP_04334033.1
81	ChlB3	*S. antibioticus*	AAZ77676
82	CalO4	*M. echinospora*	AAM70354
83	AviN	*S. viridochromogenes*	AAK83178
84	PlaP2	*S. sp. Tu6071*	ABB69750
85	CouN2	*S. rishiriensis*	AAG29787
86	CloN2	*S. roseochromogenes*	AAN65231
	KS type I PKS		
87	Plu1885	*P. luminescens*	NP_929153
88	NanA8	*S. nanchangensis*	AAP42874
89	EryAII	*S. erythraea*	YP_001102990
90	TylGI KSQ	*S. fradiae*	AAB66504
91	MerA	*S. violaceusniger*	ABJ97437
92	TamAI	*S.* sp. 3079	ADC79637
93	OleAI KSQ	*S. antibioticus*	AAF82408
94	HedT	*S. griseoruber*	AAP85336
	Closest BLAST-P hits for XclF		
95	3-Oxoacyl-ACP synthase	*R. blandensis*	WP_008043745.1

96	3-Oxoacyl-ACP synthase	*X. nematophila*	YP_003714026.1
97	3-Oxoacyl-ACP synthase	*X. nematophila*	WP_010848687.1
98	3-Oxoacyl-ACP synthase	*M. sp. PE36*	WP_006034384.1
99	3-Oxoacyl-ACP synthase	*P. profundum*	YP_132684.1
100	3-Oxoacyl-ACP synthase	*P. damselae*	WP_005305524.1
101	3-Oxoacyl-ACP synthase	*P.* sp. AK15	WP_007465048.1
102	3-Oxoacyl-ACP synthase	*P. leiognathi*	WP_008989540.1
103	3-Oxoacyl-ACP synthase	*P.* sp. SKA34	WP_006644045.1
104	3-Oxoacyl-ACP synthase	*P. angustum*	WP_005364526.1
	FabF		
105	FabF	*M.* sp. 4-46	YP_001771620
106	FabF	*C. pinensis*	ACU62401
107	cpin1855	*C. pinensis*	YP_003121552
108	Dfer_1997	*D. fermentans*	YP_003086385
109	FabB	*A. pleuropneumoniae*	ZP_00134992
110	FabB	*C.* sp. 30_2	ZP_04562837
111	NP_416826	*E. coli*	NP_416826
112	FabB	*S. boydii*	YP_001881145
	Type II PKS KS β		
113	NP_344945	*S. pneumoniae*	NP_344945
114	FabF	*T. thermophilus*	YP_143679
115	FabF	*N. punctiforme*	YP_001867862
116	FabF	*B. subtilis*	NP_389016
117	NP_645683	*S. aureus*	NP_645683
118	FabF	*P. luminescens*	NP_930065
119	FabF	*E. albertii*	ZP_02902779.1
120	FabF	*E. coli*	NP_287229
121	NP_415613	*E. coli*	NP_415613
122	FabF	*S. avermitilis*	BAC70003
	Type II PKS KS α		
123	SimA2	*S. antibioticus*	AF324838_4
124	TcmL	*S. glaucescens*	AAA67516
125	EncB	*S. maritimus*	AAF81729
126	ActIA	*S. coelicolor* A3(2)	SCO5087
127	NcnB	*S. arenae*	AAD20268
	FabB		
128	AntD (Plu4191)	*P. luminescens*	NP_931374
129	EncA	*S. maritimus*	AAF81728
130	ActiB	*S. coelicolor* A3(2)	SCO5088
131	NcnA	*S. arenae*	AAD20267
132	TcmK	*S. davawensis*	CCK26894
133	SimA1	*S. antibioticus*	AAK06784
	DarB		
134	O3I_37171	*N. brasiliensis*	ZP_09843377
135	M446_0174	*M.* sp. 4-46	YP_001767187
136	cpin6850	*C. pinensis*	YP_003126452
137	BFO_3187	*T. forsythia*	YP_005015826
138	NiasoDRAFT_0547	*N. soli*	ZP_09632794
139	Mucpa_6793	*M. paludis*	ZP_09618305
140	Oweho_0889	*O. hongkongensis*	YP_004988545
141	CHU_0390	*C. hutchinsonii*	YP_677020
142	Fluta_1447	*F. taffensis*	YP_004344279
143	Dfer_5797	*D. fermentans*	YP_003090150
144	BZARG_2045	*B. argentinensis*	ZP_08820341
145	Lacal_2074	*L.* sp. 5H-3-7-4	YP_004580348
146	Aeqsu_0932	*A. sublithincola*	YP_006417450
147	Zobellia_2074	*Z. galactanivorans*	YP_004736513
148	Lbys_1508	*L. byssophila*	YP_003997574
149	HMPREF0204_10987	*C. gleum*	ZP_07085127

150	PMI13_02465	*C.* sp. CF314	ZP_10726507
151	HMPREF0156_01383	*B. taxon* 274 str. F0058	ZP_06983320
152	HMPREF9071_0527	*C. taxon* 338 str. F0234	ZP_08201061
153	CAPGI0001_0843	*C. gingivalis*	ZP_04056582
154	HMPREF1154_2288	*C.* sp. CM59	ZP_10880679
155	HMPREF1320_1701	*C. taxon* 335 str. F0486	EJF37460
156	HMPREF1321_1154	*C. taxon* 412 str. F0487	ZP_10366882
157	CAPSP0001_1216	*C. sputigena*	ZP_03390203
158	Coch_0547	*C. ochracea*	YP_003140666
159	HMPREF1319_0374	*C. ochracea*	EJF43732
160	HMPREF1977_1456	*C. ochracea*	ZP_07866642
161	Weevi_1554	*W. virosa*	YP_004238832.1
162	HMPREF9716_01579	*M. odoratimimus*	EKB07937
163	Myrod_1723	*M. odoratus*	ZP_09672239
164	HMPREF9711_01694	*M. odoratimimus*	EKB04829
165	HMPREF9712_01161	*M. odoratimimus*	ZP_09523568
166	Fcol_11845	*F. columnare*	YP_004942963
167	FP2279	*F. psychrophilum*	YP_001297136
168	PMI10_02641	*F.* sp. CF136	ZP_10730768
169	FF52_12311	*F.* sp. F52	ZP_10481912
170	Fjoh_1102	*F. johnsoniae*	YP_001193454
171	FJSC11DRAFT_3961	*F.* sp. JSC-11	ZP_08987753
172	MICAG_1820011	*M. aeruginosa*	CCI22605
173	DP1817	*D. psychrophila*	YP_065553
174	DaAHT2_1139	*D. alkaliphilus*	YP_003690456
175	MldDRAFT_4065	delta proteobacterium MLMS-1	ZP_01289639
176	CBGD1_514	*S. gotlandica*	ZP_05070248
177	SMGD1_1386	*S. gotlandica*	EHP29910
178	Sdel_2118	*S. deleyianum*	YP_003305165
179	Sulba_2257	*S. barnesii*	YP_006405107
180	Arnit_2310	*A. nitrofigilis*	YP_003656468
181	HMPREF9401_0244	*A. butzleri*	ZP_07890833
182	Hbal_2902	*H. baltica*	YP_003061270
183	ParcA3_010100003428	*P. arctica*	ZP_10280196
184	PspoU_010100018642	*P. spongiae*	ZP_10300425
185	PSJM300_17945	*P. stutzeri*	AFN79642
186	MDS_0597	*P. mendocina*	YP_004378380
187	Psefu_0435	*P. fulva*	YP_004472512
188	Plu2164	*P. luminescens*	NP_929424
189	PA-RVA6-3077	*P. asymbiotica*	CAR66906
190	PAU_02401	*P. asymbiotica*	YP_003041237
191	PchlO6_4243	*P. chlororaphis*	ZP_10172862
192	DarB	*P. chlororaphis*	AAN18032
193	Pchl3084_3967	*P. chlororaphis*	EJL05977
194	PMI20_00702	*P.* sp. GM17	ZP_10707840
195	Daro_2368	*D. aromatica*	YP_285574
196	azo0292 DarB	*A.* sp. BH72	YP_931796
197	Rfer_3974	*R. ferrireducens*	YP_525203
198	Slit_0359	*S. lithotrophicus*	YP_003522988
199	PMI12_02025	*V. sp. CF313*	ZP_10567997
200	Vapar_3389	*V. paradoxus*	YP_002945272
201	Varpa_2231	*V. paradoxus*	YP_004154548
202	COI_2002	*M. haemolytica*	ZP_05992665
203	COK_0379	*M. haemolytica*	ZP_05988513
204	HMPREF9417_0595	*H. parainfluenzae*	ZP_08147854
205	HMPREF9952_1824	*H. pittmaniae*	ZP_08755481
206	HMPREF9064_0174	*A.segnis*	ZP_07888807
207	ATCC33389_0196	*A. aphrophilus*	EGY32238
208	NT05HA_1737	*A. aphrophilus*	YP_003008155
209	HMPREF9335_01583	*A. aphrophilus*	EHB89432

210	GCWU000324_02596	*K. oralis*	ZP_04603113
211	EIKCOROL_00456	*E. corrodens*	ZP_03712789
212	HMPREF9371_1043	*N. shayeganii*	ZP_08886538
213	HMPREF9370_1914	*N. wadsworthii*	ZP_08940206
214	NEIFLAOT_02523	*N. flavescens*	ZP_03720660
215	HMPREF0604_01363	*N. mucosa*	ZP_07993739
216	NEIFL0001_0036	*N. flavescens*	ZP_04757628
217	NEISUBOT_03200	*N. subflava*	ZP_05983976
218	NEISICOT_02133	*N. sicca*	ZP_05318975
219	HMPREF9418_1128	*N. macacae*	ZP_08684521
220	HMPREF1051_1749	*N. sicca*	EIG27057
221	HMPREF1028_00835	*N.* sp. GT4A_CT1	ZP_08888860
222	HMPREF9016_01947	*N.* taxon 014 str. F0314	ZP_06980826
223	WP_019975306.1	*Empedobacter brevis*	WP_019975306.1
224	WP_023570457.1	*Flavobacterium cauense*	WP_023570457.1
225	WP_023573188.1	*Flavobacterium enshiense*	WP_023573188.1
226	WP_026980395.1	*Flavobacterium suncheonense*	WP_026980395.1
227	WP_023575682.1	*Flavobacterium saliperosum*	WP_023575682.1
228	WP_025571904.1	*Flavobacterium* sp. JGI 0001001-D01	WP_025571904.1
229	WP_017496912.1	*Flavobacterium* sp. WG21	WP_017496912.1
230	WP_026990035.1	*Flavobacterium subsaxonicum*	WP_026990035.1
231	WP_027392755.1	*Aquimarina latercula*	WP_027392755.1
232	WP_029271432.1	*Flavobacterium sp. KJJ*	WP_029271432.1
233	WP_028979582.1	*Sporocytophaga myxococcoides*	WP_028979582.1
234	WP_026450863.1	*Aequorivita capsosiphonis*	WP_026450863.1
235	WP_027374092.1	*Chryseobacterium* sp. UNC8MFCol	WP_027374092.1
236	WP_027378929.1	*Chryseobacterium daeguense*	WP_027378929.1
237	WP_019944308.1	*Dyadobacter beijingensis*	WP_019944308.1
238	WP_016870031.1	*Fischerella muscicola*	WP_016870031.1
239	WP_026631596.1	*Dyadobacter alkalitolerans*	WP_026631596.1
240	WP_026309622.1	*Niabella aurantiaca*	WP_026309622.1
241	WP_027412514.1	*Aquimarina muelleri*	WP_027412514.1
242	WP_028121069.1	*Epilithonimonas tenax*	WP_028121069.1
243	WP_028786430.1	*Terrimonas ferruginea*	WP_028786430.1
244	WP_024771996.1	*Aquimarina macrocephali*	WP_024771996.1
245	WP_027419181.1	*Crocinitomix catalasitica*	WP_027419181.1
246	WP_025667393.1	*Aquimarina megaterium*	WP_025667393.1
247	WP_021644787.1	*Bacteroides pyogenes*	WP_021644787.1
248	AGY53864.1	*Bacteroidales bacterium CF*	AGY53864.1
249	WP_021071162.1	*Sphingobacterium paucimobilis*	WP_021071162.1
250	WP_023847326.1	*Porphyromonas gingivalis*	WP_023847326.1
251	WP_015215107.1	*Anabaena cylindrica*	WP_015215107.1

Table S6: LuxR-like proteins used for the phylogenetic tree. Sequences are ordered according to the phylogenetic tree from top to bottom.

	Protein	Accession number
1	*P. chlororaphis* O6 LuxR-like III	WP_009050747.1
2	*P. chlororaphis subsp. aurantiaca* PB-St2 LuxR-like II	ETD40528.1
3	*P. savastanoi* AhlR	WP_004667792.1
4	*P. corrugata* PcoR	WP_024779118.1
5	*V. fischeri* LuxR	AAQ90208.1
6	*V. paradoxus* EPS LuxR-like I	WP_013543355.1
7	*M.* sp. 4-46 LuxR-like I	WP_012335163.1
8	*H. baltica* ATCC 49814 LuxR-like I	WP_015826629.1
9	*A. tumefaciens* TraR	WP_010892389.1
10	*P. fluorescens* PsoR	WP_014717607.1
11	*P. syringae* LuxRI	WP_004656728.1
12	*R.* sp. Y9602 LuxR-like I	WP_013577865.1
13	*S. meliloti* NesR	WP_018096762.1
14	*R. rubrum* LuxR-like I	WP_011390028.1
15	*X. campestris* XccR	WP_011037943.1
16	*X. campestris* LuxR-like I	WP_011269597.1
17	*X. oryzae* OryR	AAR91700.1
18	*X. axonopodis* XagR	WP_029829276.1
19	*P. fluorescens* MupR	AAK28504.1
20	*P. aeruginosa* LasR	WP_003082999.1
21	*P. putida* PpuR	AAZ80478.1
22	*P. aeruginosa* QscR	WP_003160097.1
23	*P. asymbiotica* PauR	WP_015836138.1
24	*P. luminescens* PluR	WP_011148637.1
25	*M. paludis* DSM 18603 LuxR-like I	WP_008504649.1
26	*P. asymbiotica* LuxR-like II	WP_012776445.1
27	*P. asymbiotica* LuxR-like I	WP_012776448.1
28	*C. gingivalis* ATCC 33624 LuxR-like II	WP_002670238.1
29	*F. psychrophilum* JIP02/86 LuxR-like I	WP_011963028.1
30	*V. paradoxus* S110 LuxR-like I	WP_015865859.1
31	*P.* sp. GM17 PMI20 LuxR-like II	WP_007923195.1
32	*P. aureofaciens* PhzR	WP_009045585.1
33	*P. chlororaphis subsp. aureofaciens* 30-84 LuxR-like II	WP_009045585.1
34	*P. chlororaphis* O6 LuxR-like II	WP_009050817.1
35	*P. chlororaphis subsp. aurantiaca* PB-St2 LuxR-like I	WP_016703601.1
36	*S. enterica* SdiA	WP_001157166.1
37	*P. aeruginosa* RhlR	WP_004351224.1
38	*P. aureofaciens* CsaR	WP_009043433.1
39	*P. chlororaphis subsp. aureofaciens* 30-84 LuxR-like I	WP_009043433.1
40	*P.* sp. GM17 PMI20 LuxR-like I	WP_007923195.1
41	*P. chlororaphis* O6 LuxR-like I	WP_009048517.1
42	*P. chlororaphis subsp. aurantiaca* PB-St2 LuxR-like III	WP_023969002.1

Table S7: Genome analysis of 116 *darB* containing bacterial species or strains, regarding structure and assembly of the *darABC* operon, and the presence of *luxR* and *luxI genes*.

Strain	*darA*	*darB*	*darC*	*luxR*	*luxI*
Aequorivita capsosiphonis DSM 23843	ORF 301 (scaffold7)	ORF 302 (scaffold7)	ORF 313 (scaffold7)	ORF 19 (scaffold15)	
Aequorivita sublithincola DSM 14238	Aeqsu_0933	Aeqsu_0932	Aeqsu_0921	Aeqsu_1957	
Aggregatibacter aphrophilus ATCC 33389	ATCC33389_0195	ATCC33389_0196	ATCC33389_0162		
Aggregatibacter aphrophilus F0387	HMPREF9335_01584	HMPREF9335_01583	HMPREF9335_01616		
Aggregatibacter aphrophilus NJ8700	NTO5HA_1736	NTO5HA_1737	NTO5HA_1701		
Aggregatibacter segnis ATCC 33393	HMPREF9064_0175	HMPREF9064_0174	HMPREF9064_0152		
Albidiferax ferrireducens T118	Rfer_3975	Rfer_3974	Rfer_3980		
Anabaena cylindrica PCC 7122	Anacy_3063	Anacy_3064			
Aquimarina latercula 2041	ORF 311 (scaffold10)	ORF 312 (scaffold10)	ORF 321 (scaffold10)		
Aquimarina macrocephali JAMB N27	ORF 69 (scaffold20)	ORF 70 (scaffold20)	ORF 81 (scaffold20)	ORF 01 (scaffold04); ORF 15 (scaffold24); ORF 1 (scaffold6)	
Aquimarina megaterium XH134	ORF 15 (scaffold24)	ORF 16 (scaffold24)	ORF 27 (scaffold24)	ORF 232 (scaffold10)	
Aquimarina muelleri DSM 19832	ORF 47 (scaffold19)	ORF 48 (scaffold19)	ORF 58 (scaffold19)		
Arcobacter butzleri JV22	HMPREF9401_0243	HMPREF9401_0244	HMPREF9401_0261	HMPREF9401_1717	
Arcobacter nitrofigilis DSM 7299	Arnit_2309	Arnit_2310	Arnit_2315		
Azoarcus sp. BH72	azo0293	azo0292	azo0285	azo0648	
Bacteroidales bacterium CF	BRDCF_p1238	BRDCF_p1237	BRDCF_p1232	BRDCF_p1580	
Bacteroides pyogenes F0041	HMPREF1981_00060	HMPREF1981_00061		HMPREF1981_00004; HMPREF1981_02474; HMPREF1981_02785	
Bacteroidetes oral t axon 274 str. F0058	ORF 67 (scaffold16)	ORF 68 (scaffold16)	ORF 53 (scaffold15)		
Bizionia argentinen sis JUB59	BZARG_2046	BZARG_2045	BZARG_2034		
Capnocytophaga gi ngivalis ATCC 33624	CAPGI0001_2416	CAPGI0001_0843	CAPGI0001_0776		
Capnocytophaga o chracea F0287	HMPREF1977_1455	HMPREF1977_1456	HMPREF1977_1722	HMPREF1977_1768; HMPREF1977_2169	
Capnocytophaga o chracea str. Holt 25	HMPREF1319_0373	HMPREF1319_0374	HMPREF1319_0525	HMPREF1319_0572;	

				HMPREF1319_0952	
Capnocytophaga sp. CM59	HMPREF1154_0138	HMPREF1154_2288	HMPREF1154_0352	HMPREF1154_2343	
Capnocytophaga sp. oral taxon 335 str. F0486	HMPREF1320_1700	HMPREF1320_1701	HMPREF1320_1086	HMPREF1320_2182	
Capnocytophaga sp. oral taxon 338 str. F0234	HMPREF9071_1849	HMPREF9071_0527	HMPREF9071_0335		
Capnocytophaga sp. oral taxon 412 str. F0487	HMPREF1321_1155	MHPREF1321_1154	HMPREF1321_2121		
Capnocytophaga sputigena ATCC 33612	CAPSP0001_1213	CAPSP0001_1216	CAPSP0001_1050	CAPSP0001_1718; CAPSP0001_0637	
Chryseobacterium daeguense 19338	ORF 333 (scaffold2)	ORF 334 (scaffold2)	ORF 300 (scaffold2)		
Chryseobacterium gleum ATCC 35910	HMPREF0204_10986	HMPREF0204_10987	HMPREF0204_10997	HMPREF0204_11867; HMPREF0204_14208	
Chryseobacterium sp. CF314	PMI13_02464	PMI13_02465	PMI13_02475	PMI13_01931; PMI13_02863; PMI13_03175	
Chryseobacterium sp. UNC8MFCol	ORF 12 (scaffold21)	ORF 13 (scaffold21)	ORF23 (scaffold21)	ORF 4 (scaffold7)	
Myroides odoratus DSM 2801	Myrod_1724	Myrod_1723	Myrod_1713	Myrod_0136	
Pseudomonas chlororaphis subsp. *aureofaciens* 30-84	Pchl3084_3968	Pchl3084_3967	Pchl3084_0478	Pchl3084_3130	Pchl3084_2449; Pchl3084_4949
Dechloromonas aromatica RCB	Daro_2367	Daro_2368	Daro_2373	Daro_3200	
Cytophaga hutchinsonii ATCC 33406	CHU_0391	CHU_0390	CHU_0385		
Flavobacterium johnsoniae UW101	Fjoh_1103	Fjoh_1102	Fjoh_1089	Fjoh_0173; Fjoh_4220	
Methylobacterium sp. 4-46	M446_0173	M446_0174			M446_5461
Dyadobacter fermentans DSM 18053	Dfer_5796	Dfer_5797	Dfer_5802		
Hirschia baltica ATCC 49814	Hbal_2903	Hbal_2902	Hbal_1310	Hbal_0785	Hbal_1825
Chitinophaga pinensis DSM 2588	Cpin_6851	Cpin_6850	Cpin_6845	Cpin_0098	
Sulfurospirillum deleyianum DSM 6946	Sdel_2119	Sdel_2118	Sdel_2124	Sdel_0795	
Desulfurivibrio alkaliphilus AHT2	DaAHT2_0003	DaAHT2_1139	DaAHT2_1123		
Sideroxydans lithotrophicus ES-1	Slit_0358	Slit_0359	Slit_0354		
Leadbetterella byssophila DSM 17132	Lbys_1509	Lbys_1508	Lbys_1496		
Variovorax paradoxus EPS	Varpa_2230	Varpa_2231	Varpa_3239	Varpa_4471	
Weeksella virosa DSM 16922	Weevi_1553	Weevi_1554	Weevi_1564		
Fluviicola taffensis DSM 16823	Fluta_1446	Fluta_1447	Fluta_1439	Fluta_3823	
Pseudomonas	MDS_0596	MDS_0597	MDS_0627		

mendocina NK-01					
Pseudomonas fulva 12-X	Psefu_0434	Psefu_0435	Psefu_0465	Psefu_1602	
Lacinutrix sp. 5H-3-7-4	Lacal_2073	Lacal_2074	Lacal_2084	Lacal_2230	
Owenweeksia hongkongensis DSM 17368	Oweho_0890	Oweho_0889	Oweho_0884	Oweho_3240	
Tannerella forsythia ATCC 43037	BFO_3186	BFO_3187	BFO_1316	BFO_1146; BFO_1208; BFO_2702	
Flavobacterium columnare ATCC 49512	FCOL_11850	FCOL_11845	FCOL_11795	FCOL_05485	
Sulfurospirillum barnesii SES-3	Sulba_2258	Sulba_2257	Sulba_2250		
Pseudomonas stutzeri DSM 10701	PSJM300_17950	PSJM300_17948	PSJM300_02845	PSJM300_17705	
Nocardia brasiliensis ATCC 700358	O3I_010630	O3I_010635	O3I_022670	O3I_041485	
Niabella soli DSM 19437	NIASO_13195	NIASO_13200	NIASO_13500		
Desulfotalea psychrophila LSv54	DP3069	DP1817	DP1850		
Crocinitomix catalasitica ATCC 23190	ORF 8 (scaffold9)	ORF 10 (scaffold9)	ORF 15 (scaffold9)	ORF 14 (scaffold44); ORF 34 (scaffold18)	
delta proteobacterium MLMS-1	MldDRAFT_3884	MldDRAFT_4065	MldDRAFT_3849		
Dyadobacter alkalitolerans DSM 23607	ORF 397 (scaffold12)	ORF 398 (scaffold12)	ORF 404 (scaffold12)	ORF 30 (scaffold4); ORF 1064 (scaffold3)	
Dyadobacter beijingensis DSM 21582	ORF 2247 (scaffold10)	ORF 2248 (scaffold10)	ORF 2257 (scaffold10)	ORF 3107 (scaffold6)	
Eikenella corrodens ATCC 23834	EIKCOROL_00268	EIKCOROL_00456	EIKCOROL_02337		
Empedobacter brevis NBRC 14943	ORF 80 (scaffold27)	ORF 81 (scaffold27)	ORF 91 (scaffold27)	ORF 195 (scaffold32)	
Epilithonimonas tenax DSM 16811	ORF 189 (scaffold2)	ORF 190 (scaffold2)	ORF 201 (scaffold2)		
Fischerella muscicola PCC 7414	ORF 27 (scaffold177)	ORF 26 (scaffold177)	ORF 12 (scaffold174)		
Fischerella sp. JSC-11	FJSC11DRAFT_3961	FJSC11DRAFT_3962			
Flavobacterium cauense R2A-7	FCR2A7T_13120	FCR2A7T_13110	FCR2A7T_13220		
Flavobacterium enshiense DK69	FEDK69T_11700	FEDK69T_11690	FEDK69T_11800		
Flavobacterium saliperosum S13	FSS13T_06230	FSS13T_06240	FSS13T_06130		
Flavobacterium sp. CF136	PMI10_02641	PMI10_02642	PMI10_02631		
Flavobacterium sp. F52	FF52_12311	FF52_12316	FF52_12246	FF52_06255; FF52_17533	
Flavobacterium sp. JGI 0001001-D01	ORF 24 (scaffold76)	ORF 23 (scaffold76)	ORF 37 (scaffold76)		
Flavobacterium sp. KJJ	ORF 830 (scaffold2)	ORF 829 (scaffold2)	ORF 842 (scaffold2)		
Flavobacterium sp. WG21	ORF 98 (scaffold15)	ORF 97 (scaffold15)	ORF 108 (scaffold15)		
Flavobacterium	ORF 88	ORF 89	ORF 98	ORF 94	

subsaxonicum DSM 21790	(scaffold7)	(scaffold7)	(scaffold7)	(scaffold27)	
Flavobacterium suncheonense DSM 17707	ORF 186 (scaffold8)	ORF 187 (scaffold8)	ORF 192 (scaffold8)		
Haemophilus parainfluenzae ATCC 33392	HMPREF9417_0596	HMPREF9417_0595	HMPREF9417_0582		
Haemophilus pittmaniae HK 85	HMPREF9952_1825	HMPREF9952_1824	HMPREF9952_0565		
Kingella oralis ATCC 51147	GCWU000324_02598	GCWU000324_02596	GCWU000324_02637		
Mannheimia haemolytica serotype A2 str. BOVINE	COK_0380	COK_0379			
Mannheimia haemolytica serotype A2 str. OVINE	COI_2001	COI_2002			
Microcystis aeruginosa PCC 9808	MICAG_1820012	MICAG_1820011			
Mucilaginibacter paludis DSM 18603	ORF 765 (scaffold7)	ORF 766 (scaffold7)	ORF 773 (scaffold7)	ORF 1985 (scaffold1); ORF 5836 (scaffold3); ORF 387 (scaffold7); ORF 1150 (scaffold1); ORF 1558 (scaffold1)	
Myroides odoratimimus CCUG 3837	HMPREF9711_01695	HMPREF9711_01694	HMPREF9711_01697	HMPREF9711_03085	
Myroides odoratimimus CCUG 10230	HMPREF9712_01160	HMPREF9712_01161	HMPREF9712_01158	HMPREF9712_02855	
Myroides odoratimimus CIP 103059	HMPREF9716_01580	HMPREF9716_01579	HMPREF9716_01569	HMPREF9716_00125; HMPREF9716_01207	
Photorhabdus luminescens subsp. *laumondii* TTO1	Plu2163	Plu2164	Plu2165	Plu0320; Plu1817; Plu4562; Plu4274; Plu4288	
Porphyromonas gingivalis ATCC 33277		PGN_0189		PGN_1373	
Capnocytophaga ochracea DSM 7271	Coch_0548	Coch_0547	Coch_0744		
Zobellia galactanivorans	zobelia_2075	zobelia_2074	zobelia_2064	zobelia_3220	
Neisseria flavescens NRL30031/H210	NEIFLAOT_02525	NEIFLAOT_02523	NEIFLAOT_01589		
Neisseria flavescens SK114	NEIFL0001_0039	NEIFL0001_0036	NEIFL0001_1239		
Neisseria macacae ATCC 33926	HMPREF9418_1131	HMPREF9418_1128	HMPREF9418_1115		
Neisseria mucosa C102	HMPREF0604_01365	HMPREF0604_01363	HMPREF0604_01385		
Neisseria shayeganii 871	HMPREF9371_1041	HMPREF9371_1043	HMPREF9371_1824		
Neisseria sicca ATCC 29256	NEISICOT_02135	NEISICOT_02133	NEISICOT_02128		
Neisseria sicca VK64	HMPREF1051_1746	HMPREF1051_1749	HMPREF1051_1674		
Neisseria sp. GT4A_CT1	HMPREF1028_00832	HMPREF1028_00835	HMPREF1028_00840		

Neisseria sp. oral taxon 014 str. F0314	ORF 66 (scaffold20)	ORF 70 (scaffold20)	ORF 82 (scaffold20)		
Neisseria subflava NJ9703	NEISUBOT_03198	NEISUBOT_03200	NEISUBOT_03174		
Neisseria wadsworthii 9715	HMPREF9370_1915	HMPREF9370_1914	HMPREF9370_1926		
Niabella aurantiaca DSM 17617	ORF 726 (scaffold5)	ORF 727 (scaffold5)	ORF 1151 (scaffold5)		
Photorhabdus asymbiotica	PAU_02402	PAU_02401	PAU_02400	PAU_00252; PAU_00255; PAU_00467; PAU_03807; PAU_04062	
Pseudoalteromonas arctica A 37-1-2	PARC_10984	PARC_10989	PARC_10954	PARC_05648; PARC_16911; PARC_133373	
Pseudoalteromonas spongiae UST010723-006	PSPO_03655	PSPO_03650	PSPO_03675	PSPO_01321; PSPO_02000; PSPO_07959	
Pseudomonas chlororaphis O6	PchlO6_4244	Pchl06_4243	Pchl06_0482	Pchl06_2663; Pchl06_3394; Pchl06_3471	Pchl06_2661; Pchl06_5139; Pchl06_5218
Pseudomonas chlororaphis subsp. *aurantiaca* PB-St2	U724_29720	U724_29715	U724_27995	U724_04375; U724_04815; U724_20385	U724_20380; U724_10400; U724_10750
Pseudomonas sp. GM17	PMI20_00701	PMI20_00702	PMI20_03176	PMI20_01529; PMI20_02078; PMI20_05272	PMI20_1530; PMI20_01270
Sphingobacterium paucimobilis HER1398	M472_06765	M472_06770	M472_06820	M472_18005	
Sporocytophaga myxococcoides DSM 11118	ORF 3606 (scaffold6)	ORF 3608 (scaffold6)	ORF 3630 (scaffold6)	ORF 16 (scaffold13); ORF 55 (scaffold13); ORF 573 (scaffold5); ORF 852 (scaffold2)	
Terrimonas ferruginea DSM 30193	ORF 2536 (scaffold10)	ORF 2540 (scaffold10)	ORF 2558 (scaffold10)		
Variovorax paradoxus S110	Vapar_3390	Vapar_3389	Vapar_2669		Vapar_5808
Variovorax sp. CF313	PMI12_02024	PMI12_02025	PMI12_0548	PMI12_00090	
Sulfurimonas gotlandica GD1	SMGD1_1387	SMGD1_1386	SMGD1_1381		
F. psychrophilum JIP02/86	FP2280	FP2279	FP2267		

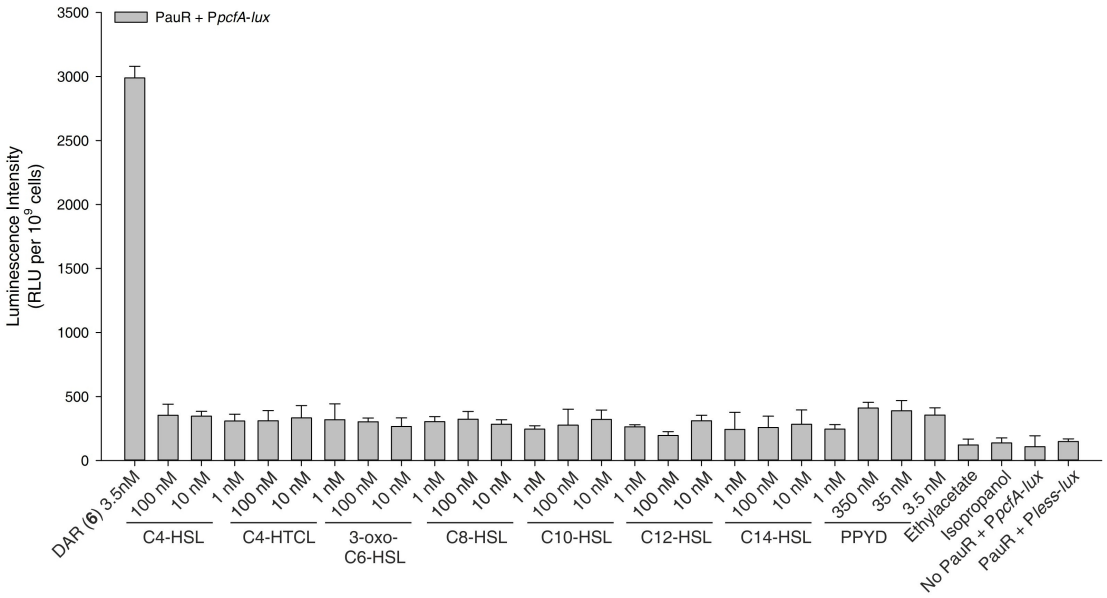

Fig. S1: PauR neither senses exogenous PPYs nor exogenous AHLs. PauR does not sense different acyl-homoserinelactones or photopyrones (PPYD), but most specifically senses 2,5-dialkylresorcinol (DAR (**6**). *E. coli* LMG194 strains harbouring a P_{pcfA}-*luxCDABE* (P_{pcfA}-*lux*) fusion as well as pBAD-*pauR* were cultivated, and exposed to 3.5 nM PPYD, to N-butyryl-DL-homoserinelactone (C4-HSL), N-butyryl-DL-homocysteinthiolactone (C4-HCTL), N-3-oxo-hexanoyl-DL-homoserinelactone (3-oxo-C6-HSL), N-octanoyl-DL-homoserinelactone (C8-HSL), N-decanoyl-DL-homoserinelactone (C10-HSL), N-dodecanoyl-DL-homoserinelactone (C12-HSL), and N-tetradecanoyl-DL-homoserinelactone (C14-HSL), respectively, in concentrations of 1 nM, 10 nM, and 100 nM, respectively. As negative controls isopropanol and ethylacetate and as positive control 3.5 nM DAR (**6**) was added to the *E. coli* LMG194 cells harbouring a P_{pcfA}-*luxCDABE* (P_{pcfA}-*lux*) fusion as well as pBAD-*pauR*. Cells with no PauR or cells harbouring a *luxCDABE* operon without a promoter (P_{less}) were used as controls as well. Error bars represent standard deviation of at least three independently performed experiments. RLU, relative light units.

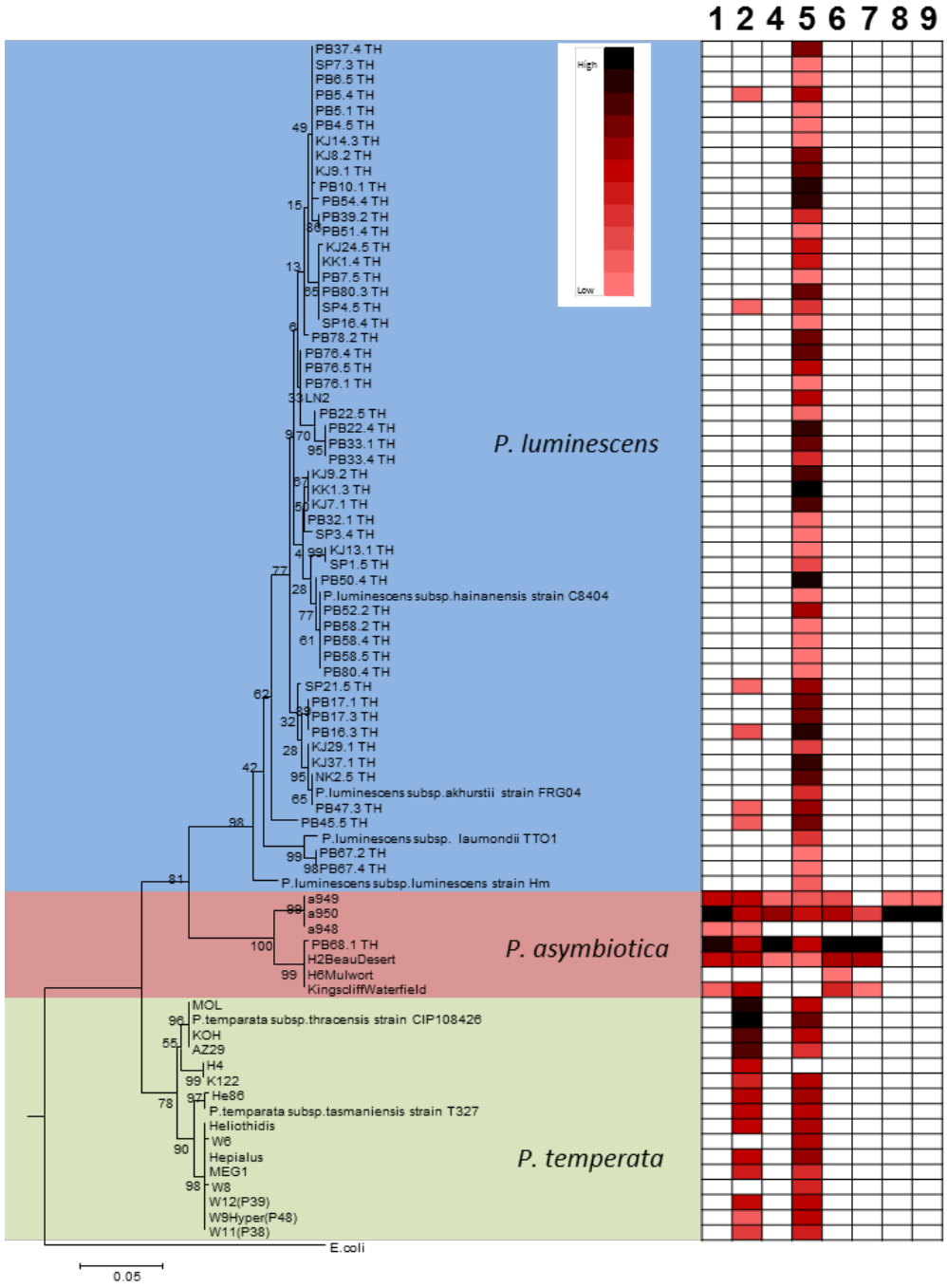

Fig. S2: Phylogeny of different *Photorhabdus* strains and their production of CHD and DAR derivatives. Numbers refer to structures in Fig. 1C. Compound **3** (missing in this figure) is only produced in trace amounts in *P. asymbiotica* strains and is only visible after derivatisation (17).

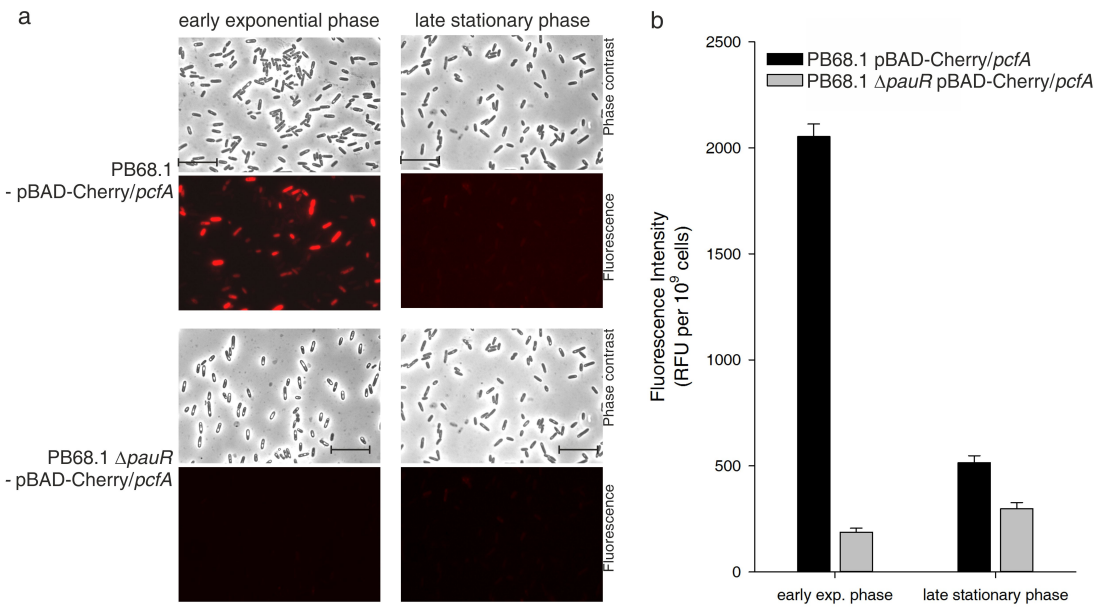

Fig. S3: Growth phase dependent P_{pcfA} promoter activity in *P. asymbiotica*. Strains *P. asymbiotica* PB68.1 and *P. asymbiotica* PB68.1 Δ*pauR* carrying plasmid pBAD-Cherry/*pcfA* were cultivated, and after 16 h (early exponential phase) as well as 48 h (late stationary phase) microscopically analyzed for fluorescence. (**a**) Upper picture: phase contrast channel; lower picture: fluorescence channel (excitation wavelength: 546 nm). The figure represents one characteristic of at least three independently performed experiments. Fluorescence was quantified (**b**) using an "Infinite 500" plate fluorimeter. Error bars represent standard deviations of at least three independently performed experiments. RFU=relative fluorescence units.

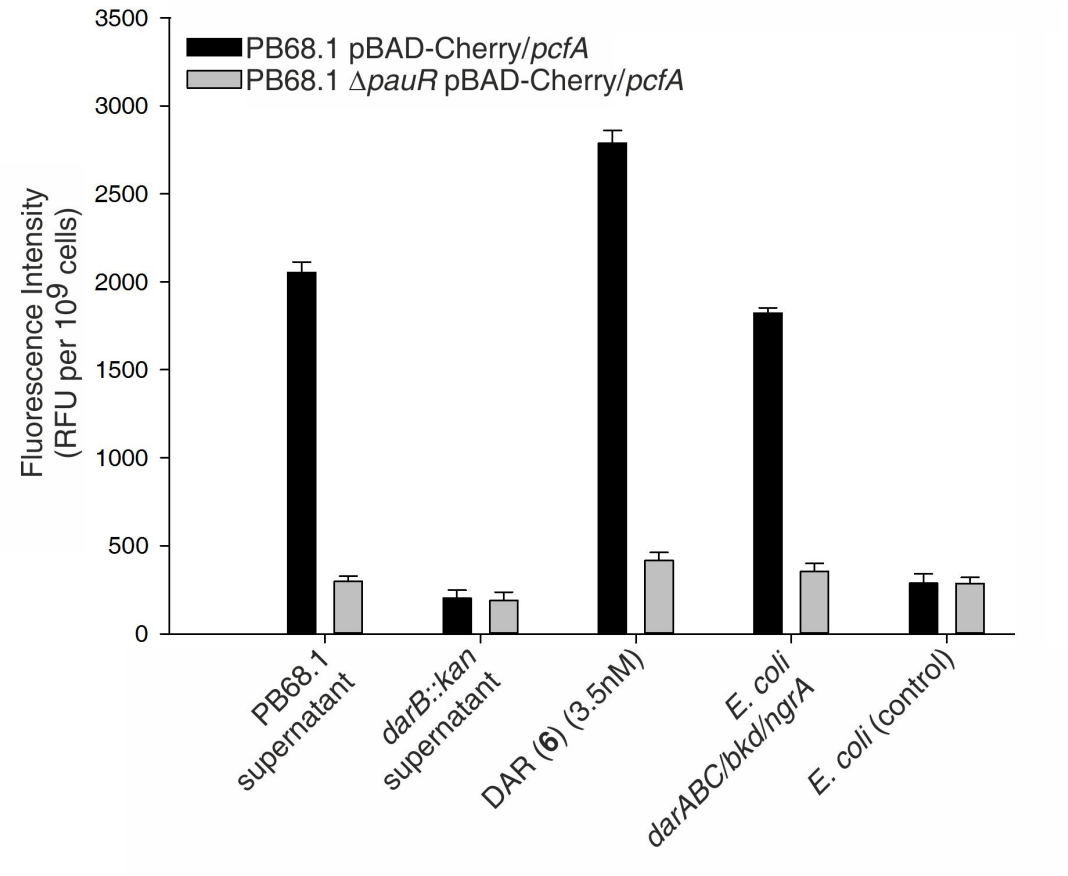

Fig. S4: Dialkylresorcinol bioactivity. *P. asymbiotica* strain PB68.1 carrying plasmid pBAD-Cherry/*pcfA* from the late stationary growth phase (P_{pcfA} promoter activity is almost off) was exposed to different extracts (PB68.1 supernatant, PB68.1 *darB::kan* supernatant, *E. coli* LMG194 expressing *darABC*/*bkd*/*ngrA* and *E. coli* LMG194 harbouring empty plasmids) or pure compounds **6** and then analyzed for fluorescence in a fluorimeter. Fluorescence was quantified using an "Infinite 500" plate fluorimeter. Error bars represent standard deviations of at least three independently performed experiments. RFU=relative fluorescence units.

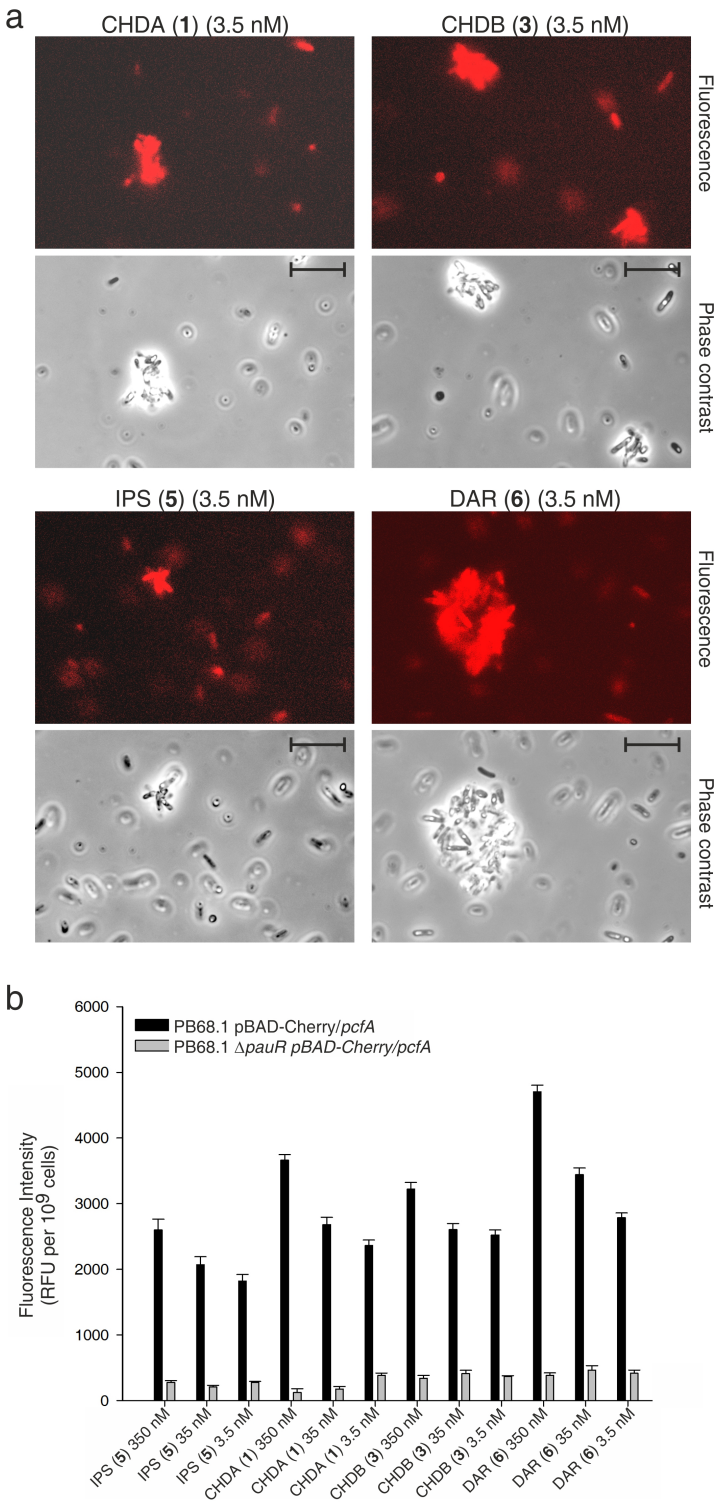

Fig. S5: Concentration-dependent P_{pcfA} induction by CHDs and DARs. *P. asymbiotica* PB68.1 strain carrying plasmid pBAD-Cherry/*pcfA* from the late stationary growth phase (P_{pcfA} promoter activity is almost off) was exposed to 350 nM, 35 nM, or 3.5 nM of **1**, **3**, **5**, and **6**, respectively. After incubation for 1 h at 37°C, fluorescence and cell clumping of the cells was analyzed in the microscope (**a**) and fluorescence was quantified using an "Infinite 500" (Tecan, Austria) plate fluorimeter (**b**). In (**a**) only concentrations of 3.5 nM of DARs and CHDs are shown. The bars indicate a scale of 10 µm. The figure represents one characteristic of at least three independently performed experiments. Error bars in (**b**) represent standard deviation of at least three independently performed experiments. RFU=relative fluorescence units.

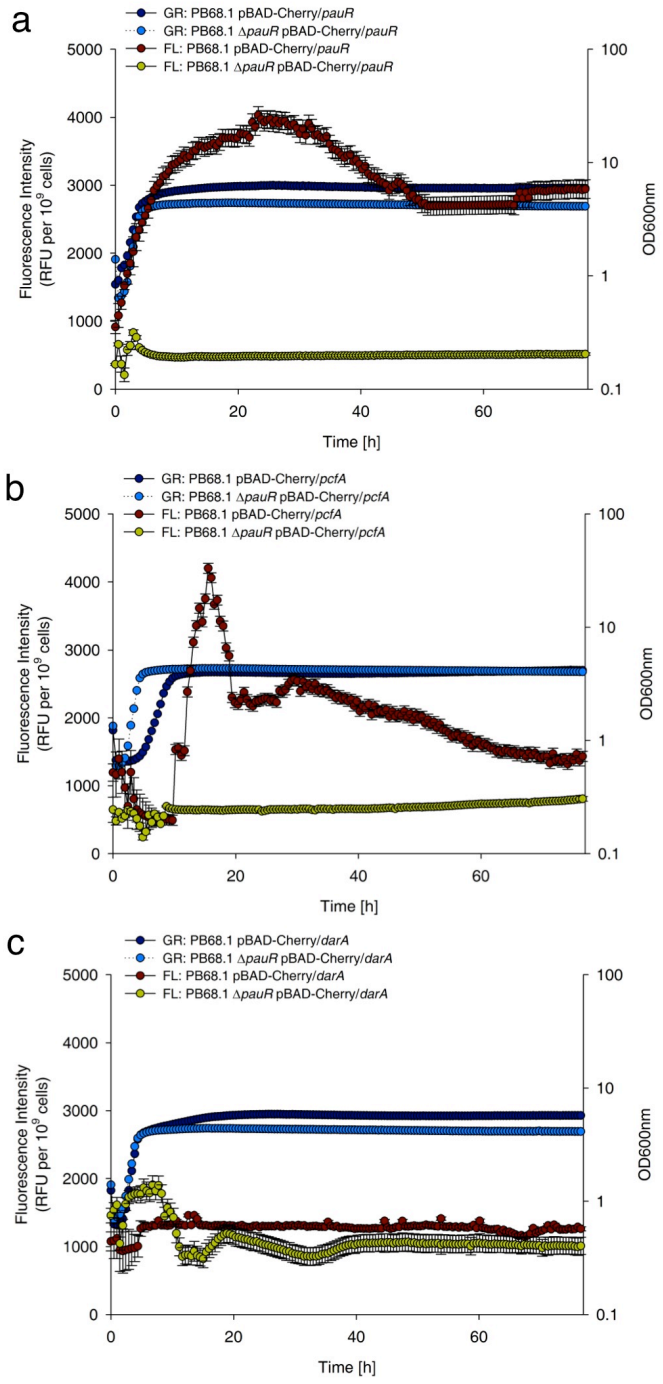

Fig. S6: Growth-curve dependent P_{pauR}, P_{pcfA}, and P_{darA} promoter activities in *P. asymbiotica* wild-type and *P. asymbiotica* ΔpauR. *P. asymbiotica* PB68.1 or *P. asymbiotica* PB68.1 Δ*pauR* carrying plasmid pBAD-Cherry/*pauR* (**a**), pBAD-Cherry/*pcfA* (**b**), or pBAD-Cherry/*darA* (**c**), respectively, was aerobically grown at 37°C for 70 hours. The growth curve (GR) was measured for each strain and fluorescence (FL) using an "Infinite 500" (Tecan, Austria) plate fluorimeter was quantified for each strain. Error bars represent the standard deviation of at least three independently performed experiments. RFU=relative fluorescence units.

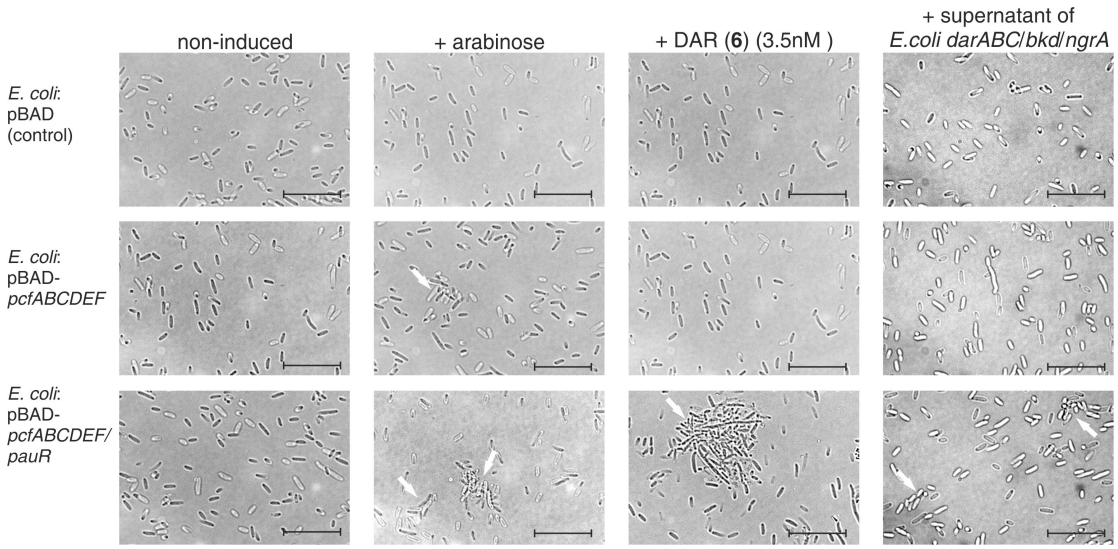

Fig. S7: Heterologous reconstruction of the DarABC/PauR cell-cell communication circuit in *E. coli*. *E. coli* LMG194 cells carrying plasmid pBAD24, pBAD-*pcfABCDEF* or pBAD-*pcfABCDEF*/*pauR* were cultivated, expression of the *pcfABCDEF* operon was induced by addition of 0.2% (w/v) arabinose or via the native promoter with 3.5 nM of **6**, and cells were analyzed for clumping by phase contrast microscopy. Additionally *E. coli* LMG194 cells carrying plasmid pBAD24, pBAD-*pcfABCDEF* or pBAD-*pcfABCDEF*/*pauR* was incubated with the supernatant of *E. coli* cells carrying *darABC/bkdABC* and *ngrA*, expression was induced with 0.2% (w/v) arabinose and 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG). Then, cells were analyzed for cell clumping (see white arrows) by phase contrast microscopy. Figures represent one of at least three independently performed experiments. Scale bars, 20 µm.

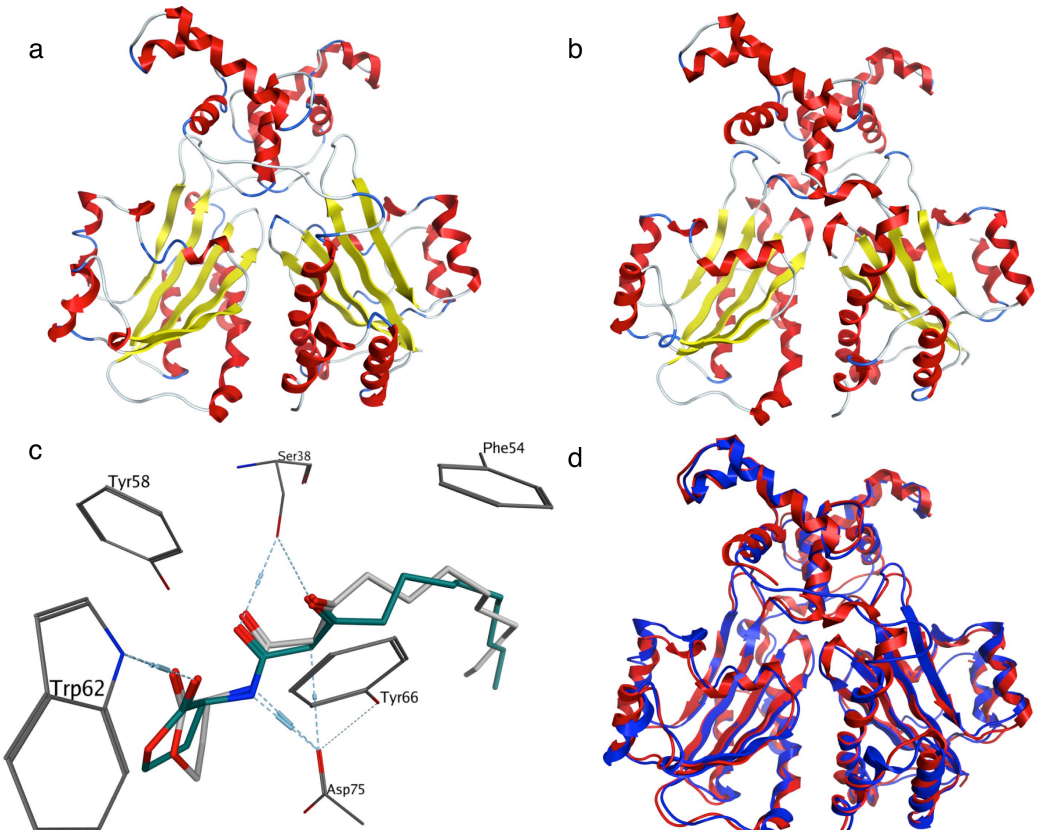

Fig. S8: 3D Modelling of PauR. Tertiary structure of the modeled PauR dimer from *Photorhabdus asymbiotica* (**a**). For the calculation of the PauR structure the tertiary structure of QscR (PDB ID: 3SZT) from *Pseudomonas aeruginosa* (**b**) was used as a template. To determine the quality of the docking procedure the co-crystallized QscR of *Pseudomonas aeruginosa* ligand (*N*-3-oxo-dodecanoyl-L-homoserine lactone) and the docked ligand (cyan) were superposed (**c**). The superoposition (**d**) of PauR (blue) and QscR (red) revealed a root-mean-square deviation (RMSD) of 1.5 Å.

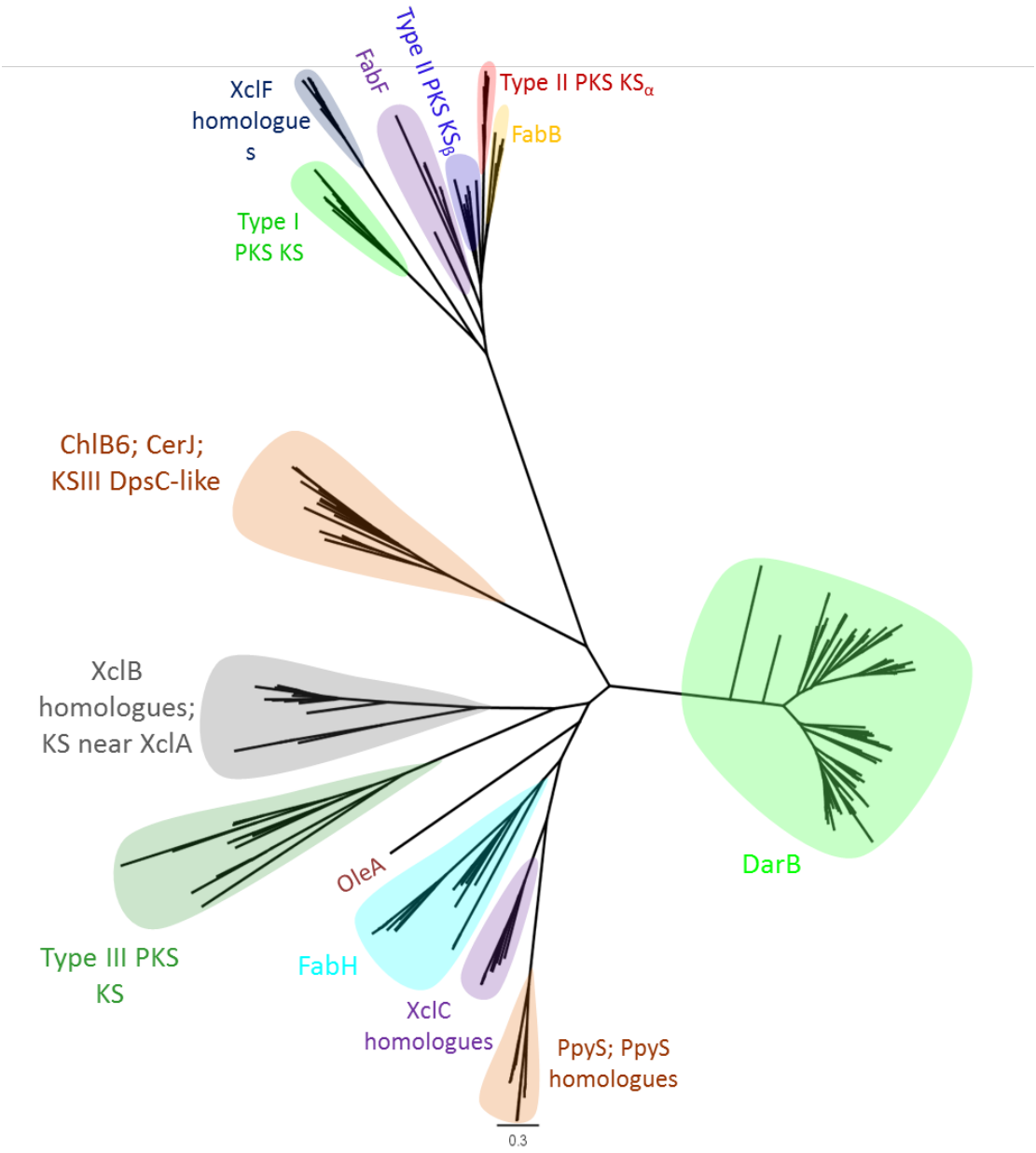

Fig. S9: Phylogenetic tree (PHYML) comprising different ketosynthases. KS sequences are listed in SI Appendix, Table S5 and a zoomed version of the DarB branch is shown for clarity in SI Appendix, Fig. S10. The scale bar indicates the degree of divergence as substitutions per sequence position.

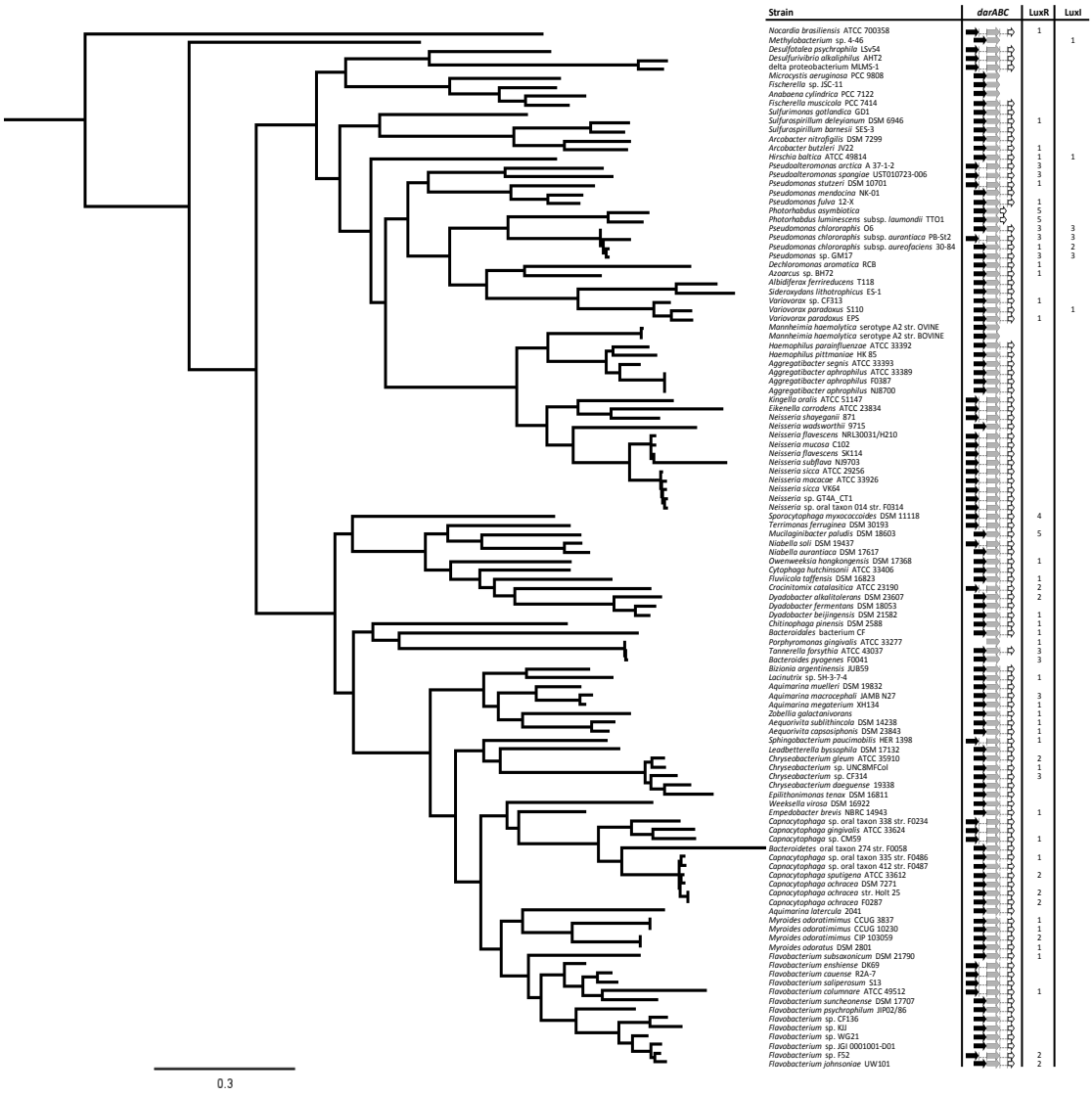

Fig. S10: Detailed phylogeny of *darB* (SI Appendix, Fig. S9), the corresponding arrangement of *darABC* in these genomes as well as the number of *luxR* and *luxI* genes identified are shown. A black arrow indicates *darA*, a grey arrow *darB* and a white arrow with a black frame *darC*. All identification numbers of identified genes are listed in SI Appendix, Table S7.

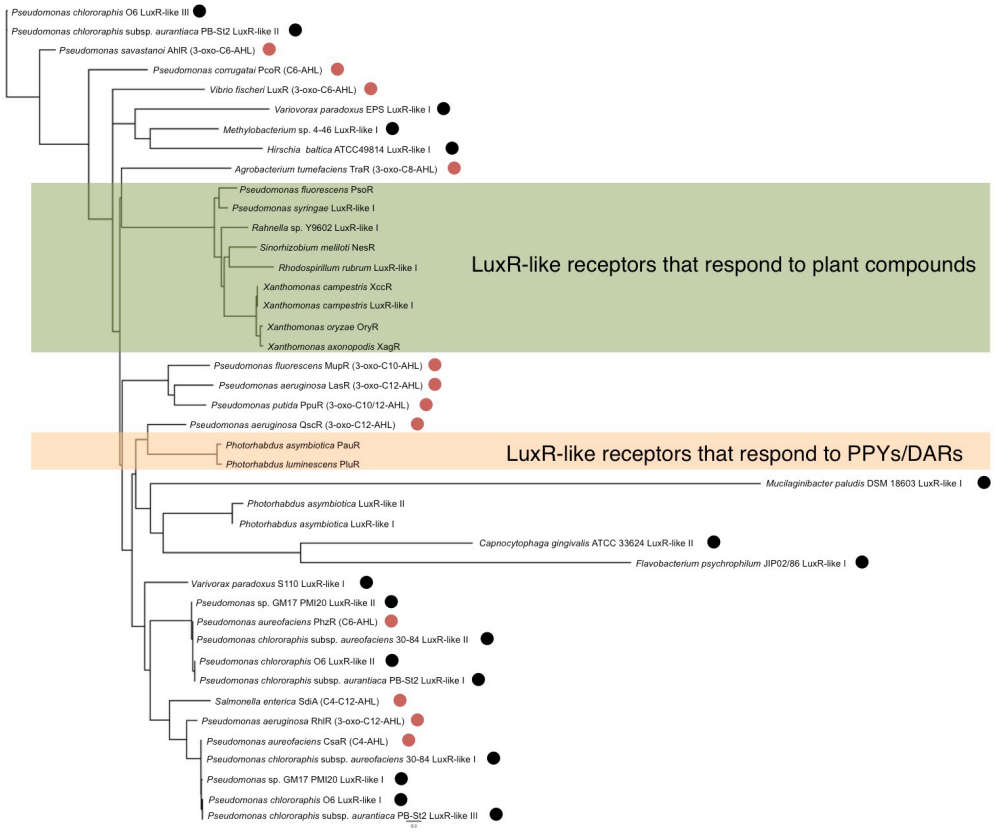

Fig. S11: Phylogenetic tree (PHYML) comprising different LuxR-like proteins. Red dots refer to known AHL-binding LuxR receptors (with the structure of the acyl side chain of their major AHL included in brackets). Black dots refer to LuxR sequences identified in DarB-containing genomes (which were also used for the generation of the KS phylogeny in SI Appendix, Fig. S9). LuxR-like proteins from plant associated bacteria (PAB) have been proposed to detect signals from plants (21). A list of all proteins and their accession numbers is provided in SI Appendix, Table S6. The scale bar indicates the degree of divergence as substitutions per sequence position.

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