SUPPLEMENTARY CONTENT

Heterogeneous regulation of bacterial natural product biosynthesis via a novel transcription factor

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Supplementary Tables

Table S1. Bacterial strains used in this study.

Strain	Genotype	Reference
E. coli DH10B	F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L *nup*G λ–	[1]
E. coli Dh5α λ*pir*	*recA1, gyrA (lacIZYA-argF) (80d lac [lacZ] M15) pir RK6*	[2]
E. coli S17-1 λpir	Tp^R Sm^R *recA*, *thi*, *pro*, *hsdR*-M+RP4: 2-Tc:Mu: Km Tn7 *λpir*	Biomedal S.L., Sevilla, Spain
E. coli ST18	*E. coli* S17 *λpir ΔhemA*	[3]
E. coli BL21 (DE3) Star	F^- *ompT hsd*S_B (r_B^- m_B^-) *gal dcm rne*131 (DE3)	Invitrogen
E. coli LMG194	F- Δ*lacX74 galE galK thi rpsL* Δ*phoA* (PvuII) Δ*ara714 leu*::Tn10	[4]
P. luminescens subsp. *laumondii* TT01-1°	Wild type 1° variant	[5]
P. luminescens subsp. *laumondii* TT01-2°	Wild type 2° variant	Lab collection, Dr. David Clarke, University College Cork
P. luminescens TT01-1°Δ*antJ*	Wild type 1° variant containing a deletion of a*ntJ*	This study
P. luminescens TT01-1°Δ*plu0918-plu0925*	Wild type 1° variant containing a deletion of *plu0918-plu0925*	This study
P. luminescens TT01-1°Δ*plu2548*	Wild type 1° variant containing a deletion of *plu2548*	This study
P. luminescens TT01-1° P_{antA}-*mCherry*	TT01-1° harboring P_{antA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	[6]
P. luminescens TT01-2° P_{antA}-*mCherry*	TT01-2° harboring P_{antA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1°Δ*antJ* P_{antA}-*mCherry*	TT01-1°Δ*antJ* harboring P_{antA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° P_{antA}-*mCherry* P_{antJ}-*eYFP*	TT01-1° harboring P_{antA}-*mCherry and* P_{antJ}-*eYFP* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-2° P_{antA}-*mCherry* P_{antJ}-*eyfp*	TT01-2° harboring P_{antA}-*mCherry and* P_{antJ}-*eYFP* reporter reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° Δ*antJ* P_{antA}-*mCherry* P_{antJ}-*eYFP*	TT01-1°Δ*antJ* harboring P_{antA}-*mCherry and* P_{antJ}-*eYFP* reporter reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study

P. luminescens TT01-1° P_{antA}-*mCherry* P_{antJ}-*antJ-eYFP*	TT01-1° harboring P_{antA}-*mCherry and* P_{antJ}-*antJ-eYFP* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-2° P_{antA}-*mCherry* P_{antJ}-*antJ-eYFP*	TT01-2° harboring P_{antA}-*mCherry and* P_{antJ}-*antJ-eYFP* reporter reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1°Δ*antJ* P_{antA}-*mCherry* P_{antJ}- *antJ-eYFP*	TT01-1°Δ*antJ* harboring P_{antA}-*mCherry and* P_{antJ}-*antJ-eYFP* reporter reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° P_{antA}-*mCherry* P_{tac}-*antJ-eYFP*	TT01-1° harboring P_{antA}-*mCherry and* P_{tac}-*antJ-eYFP* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-2° P_{antA}-*mCherry* P_{tac}-*antJ-eYFP*	TT01-2° harboring P_{antA}-*mCherry and* P_{tac}-*antJ-eYFP* reporter reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° Δ*antJ* P_{antA}-*mCherry* P_{tac}- *antJ-eYFP*	TT01-1°Δ*antJ* harboring P_{antA}-*mCherry and* P_{tac}-*antJ-eYFP* reporter reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° P_{less}-*mCherry* P_{less}-*eYFP*	TT01-1°harboring P_{less}-*mCherry and* P_{less}-*eYFP* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-2° Pless-mCherry Pless-eYFP	TT01-2° harboring Pless-mCherry and Pless-eYFP reporter integrated at the rpmE/glmS site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1°Δ*antJ* Pless-mCherry Pless-eYFP	TT01-1°Δ*antJ* harboring Pless-mCherry and Pless-eYFP reporter integrated at the rpmE/glmS site, Kan^R, $Gent^R$	This study

Table S2. Plasmids used in this study.

Plasmid	Genotype	Reference
pJet1.2/blunt	Cloning vector	Thermo Scientific
pCKcipB	pDS132 base vector , R6K ori; Cm^R; oriT; *sacB*; relaxase *traI,* additional *Bgl*II within the mcs	[7]
pACYCDuet-1	Expression vector, p15A ori, Cm^R	Merck (Darmstadt)
pCOLADuet-1	Expression vector, ColA ori, Km^R	Merck (Darmstadt)
pACYC_tacI/I	pACYC-Duet1 based vector, p15A ori, Cm^R, 2 msc with *tacI* promoters	[8]
pCOLA_tacI/I	pCOLA-Duet1 based vector, ColA ori, Km^R, 2 mcs with *tacI* promoters	[8]
pCATI4	expression casette inserted in pCOLADuet-1, ColA ori, Km^R, His_6-tag	T. Schöner, Frankfurt unpublished
pAKH01	R6K ori, Cm^R, oriT, *sacB*, relaxase *traI,* encoding the 926 bp upstream and the 788 bp downstream region of *antJ*	This study
pAKH02	R6K ori, Cm^r, oriT, *sacB*, relaxase *traI,* encoding the 843 bp upstream and the 733 bp downstream region of *plu0918-0925*	This study
pAKH03	R6K ori, Cm^r, oriT, *sacB*, relaxase *traI,* encoding the 853 bp upstream and the 912 bp downstream region of plu*2548*	This study
pAKH04	ColA ori, Km^R, tacI promoter, *antJ*	This study
pAKH05	p15A ori, Cm^R, tacI promoter, *antJ*	This study
pBAD24	Expression vector, arabinose inducible promoter, ApR	[4]
pBAD24-antJ	*antJ* in pBAD24	This study
pBAD24-antJ-His	*antJ* in pBAD24 with C-terminal His-tag	This study
pCATI4_antJ	*antJ* with C-terminal His-tag in pCATI4	This study
pBBR1-mcs5-tt-lux	*luxCDABE* and terminators lambda T0 rrnB1 T1 cloned into pBBR1-mcs5 for plasmid-based transcriptional fusions; Gm^R	[9]
pBBR1-PantA-lux	*luxCDABE* under the control of the *antA*-promoter	This study
pBBR1-PantA-s1-lux	*luxCDABE* under the control of the truncated promoter construct P_{antA}-S1	This study
pBBR1-PantA-s2-lux	*luxCDABE* under the control of the truncated promoter construct P_{antA}-S2	This study
pBBR1-PantA-s3-lux	*luxCDABE* under the control of the truncated promoter construct P_{antA}-S3	This study
pBBR1-PantA-mm1-lux	*luxCDABE* under the control of the promoter construct P_{antA}-mm1 with an altered binding motif 1	This study

pBBR1-PantA-mm2-lux	*luxCDABE* under the control of the promoter construct P_{antA}-mm2 with an altered binding motif 2	This study
pBBR1-PantA-mspacer-lux	*luxCDABE* under the control of the promoter construct P_{antA}-mspacer with an altered spacer region	This study
pPINT-mCherry	Km^R, Gm^R and *mCherry* in pPINT	[6]
pPINT-P_{antA}-mCherry	Km^R, Gm^R, *antA*-promoter upstream of *mCherry*	[6]
pEYFP	Ap^R, *lac*-promoter upstream of *eYFP*	Takara-Clonetech, Saint-Germain-en-Laye, France)
pPEINT-mCherry-eYFP	Km^R, Gm^R, *mCherry* and eYFP in pPINT	This study
pPEINT-P_{antA}-mCherry-P_{antJ}-eYFP	Km^R, Gm^R, *antA*-promoter upstream of *mCherry, antJ*-promoter upstream of *eYFP*	This study
pPEINT-P_{antA}-mCherry-P_{antJ}-*antJ*-eYFP	Km^R, Gm^R, *antA*-promoter upstream of *mCherry, antJ*-promoter upstream of *antJ-eYFP*	This study
pPEINT-P_{antA}-mCherry-P_{tac}-*antJ*-eYFP	Km^R, Gm^R, *antA*-promoter upstream of *mCherry, tac*-promoter upstream of *antJ-eYFP, lacI*	This study
pPEINT-P_{antJ}-mCherry-Pless-eYFP	Km^R, Gm^R, *antJ* promoter upstream of *mCherry*	This study
pPEINT-P_{antJ}-antJ-mCherry-Pless-eYFP	Km^R, Gm^R, *antJ* promoter upstream of antJ-*mCherry*	This study

Table S3. Oligonucleotides used in this study.

Name	Sequence (5'-3')
AKHp01	[Btn]ACATTATATACCTTATGGATTTCAAGATG
AKHp02	GAAAACCTGAAAGCTTCTATTACC
AKHp03	[Btn]TGAAGGTAAGAGTACGTGTTTGG
AKHp04	GGCATATTTCATCTGAACTATTCC
AKHp05	[Btn]GTATAGTGACATGATAAAGTGTTTTGG
AKHp06	ATATCTGCAGTATACATCGCGATGGTAAGG
AKHp07	ATCATTTATTTCCGAACTCCTCATTTTAATAA
AKHp08	AGTTCGGAAATAAATGATTACGGATGAGTAATTTATG
AKHp09	ATATGGATCCAAATGATTATTGGCAATGGAC
AKHp10	GGCGGTAGCTATATTTGATGG
AKHp11	CATTTTCCGATTCACAATTAACC
AKHp12	CAGCTTAATTAACCTAGGCTGCTG
AKHp13	GGAATTCCCTCCTGTGT
AKHp14	CAATTTCACACAGGAGGGAATTCCATGTCAAGAACCGAAAGAC
AKHp15	CAGCAGCCTAGGTTAATTAAGCTGCATCCGTAATCATTTATTTAGG
AKHp16	CCTCTAGAGTCGACCTGCAGAAATCTGGCGAATTAACTTACC
AKHp17	AGTGGTTCATTGATCATGATTAAACTGTAAAACTGTAA
AKHp18	TTTAATCATGATCAATGAACCACTCCGTCAC
AKHp19	TCCCGGGAGAGCTCAGATCTTAGTATTCCGCACCAGTGC
AKHp20	ACGGGTATTTAAACATTAACAAGG
AKHp21	CCTATGGATTTCAAGATACATCG
AKHp22	CCTCTAGAGTCGACCTGCAGAATTTCACTGGATGTTCACG
AKHp23	GCGTAGATGAATTATATAACTTGTGGCTAAAATAATTTC
AKHp24	CCACAAGTTATATAATTCATCTACGCGGAGAGG
AKHp25	TCCCGGGAGAGCTCAGATCTCAAGGCACAAAATATCAAGG
AKHp26	AATACATGATAGCCAAGCTTCC
AHKp27	AACTGGTAAAAGAGTTTGACGG
AKHp28	[Cy3]TGTGTATTTAGCGGTTATCG
AKHp29	[Cy3]TCAAACACCAATCTATCACG
AKHp30	TGTGTATTTAGCGGTTATCGGTATAGTGACATGATAAAGTGTTTTGG
AKHp31	TCAAACACCAATCTATCACGTTACGAAAAATATTAACCTTTTCACG
AKHp32	TGTGTATTTAGCGGTTATCGACACCGATATATTATTGTTCTTTACG
AKHp33	TCAAACACCAATCTATCACGTTACCCCTAACAAGCATTATTC
AKHp34	TGTGTATTTAGCGGTTATCGATGCTGACAAAAACTGTCAC
AKHp35	TCAAACACCAATCTATCACGATCTTGAAATCTATAGGGTATATATTATTGC
AKHp36	TGTGTATTTAGCGGTTATCGTCGTGTTTTTCCGTGAATAGC
AKHp37	TCAAACACCAATCTATCACGCACATCGTTATCTATGCTACCG
AKHp38	TGTGTATTTAGCGGTTATCGTGTATCATGACGGCAAGG
AKHp39	TCAAACACCAATCTATCACGATTTATACCCGTCATCTTTCAAG
AKHp40	TGTGTATTTAGCGGTTATCGACCGAGTGCGGCCAACAAAG
AKHp41	TCAAACACCAATCTATCACGCCTGTCGCGCCGGTAATAAAGG
AKHp42	ACTAGTCATCATCACCACCATC

AKHp43	TGCCATGGTATATCTCCTTATTAAAG
AKHp44	TAAGGAGATATACCATGGCATCAAGAACCGAAAGACTTATCG
AKHp45	TGGTGGTGATGATGACTAGTGGATAAATTATTATCAGACAATCCC
antJ-NheI_fwd	GCGGCTAGCAGGAGGAATTCATGTCAAGAACCGAAAGA
antJ-ostop-NdeI_rev	GCGCATATGGGATAAATTATTATCAGACAA
antJ-NdeI_rev	GCGCATATGGGATAAATTATTATCAGACAA
pBAD24-Seq_fwd	GCCGTCACTGCGTCTTTTACTGG
pBAD24-Seq_rev	CGCTACGGCGTTTCACTTCTG
PantA-XbaI_fwd	GCGTCTAGATAATGCAGAAATTATTGCT
Pant-XmaI_rev	GCGCCCGGGCTGAACTATTCCTATCGTTA
PantA-s1-XbaI_fwd	GCGTCTAGACGTAAATGCTGACAAAAACT
PantA-s2_fwd	GCGTCTAGATTTCGTAAGACAAAAACTGTCACTAATT
PantA-s3-XbaI_fwd	GCGTCTAGAATGCTTGTTAGGGGTAATC
PantA-mm1-ol_fwd	TTTCGTAACGTAGGACAAAAACTGT
PantA-mm1-ol_rev	ACAGTTTTTGTCCTACGTTACGAAA
PantA-mm2_fwd	TTATGAATACGTAGTGTTAGGGGTAAT
PantA-mm2_rev	ATTACCCCTAACACTACGTATTCATAA
PantA-spacer-ol_rev	TGTCACTGGGGGTGAAGCATTTACGAAAAA
PantA-spacer-ol_fwd	TCACCCCCAGTGACAGCCGGGCGTCCGAATGCTTGTTAGGGGTA
check-pBBR_fwd	GGAGCTTGCGGCCCGGACG
check-pBBR_rev	GTCATATTTGCCCTCCTGG
PantA-Btn_fwd	(Btn)TAATGCAGAAATTATTGCT
eyfp-mcs_fwd	GCTGGGCCCGAGCTCGCGGCCGCCCACCATGGTGAGCAAG
eyfp-EcoRI_rev	GCTGAATTCTTACTTGTACAGCTCGTCC
PantJ-NheI_fwd	GCTGCTAGCTGATAAGTTATCTTGTTGA
Ptac-antJ-NheI_fwd	GCTGCTAGCCATTAATTGCGTTGCGCTCA
PantJ-NotI_rev	GCTGCGGCCGCTTCCGAACTCCTCATTTTA
antJ-ostop-NcoI_rev	GCTCCATGGAGGATAAATTATTATCAGACA
check-eyfp2_rev	GTTTGGATGGATATCTGGT
check-mcherry-ins_rev	GGCCTTCCTTCTCCTTCAC
PantA-NheI_fwd	GCTGCTAGCTAATGCAGAAATTATTGCT
PantA-NheI_rev	GCGGCTAGCCTGAACTATTCCTATCGTTA
PantJ-BamHI_rev	GCGGGATCCTTCCGAACTCCTCATTTTA
antJ-ostop-	GCGGGATCCGGATAAATTATTATCAGACA

BamHI_rev	
check-eyfp_rev	CTGCAGGCCGTAGCCGAA
check-rpmE_fwd	CTCCCAAATAAAGTTTAGG
check-glmS_rev	GTACGTGAATCTGATTTTG
oriT_fwd	CAGGGTTATGCAGCGGAAA
gmR-pNPTS_fwd	GATAAGCTGTCAAACATGAGAGTAGCGTATGCGCTCAC

[Btn]: 5´- biotinylation, [Cy3]: 5´-Cy3-label

Table S4: Summary of differently expressed (mRNA) transcripts (sense direction) in the TT01-1°Δ*antJ* strain. Listed are the fold changes in read counts of TT01-1°Δ*antJ,* TT01-1°Δ*antJ* + *antJ*$^{+}$ and TT01-1° + control1 compared to TT01-1°. The false discovery rate (FDR) is < 0.01. For clarity fold changes < 1 are represented as –(fold change)$^{-1}$. The functions of genes without a given gene name were domain guided annotated using the NCBI tools: protein blast and conserved domain search.

Locus tag	Gene	Putative function of the gene product	Fold change in read counts compared to the WT		
			TT01-1° Δ*antJ*	TT01-1° Δ*antJ* + *antJ*$^{+}$	TT01-1° + control1
plu0033		tail assembly protein	-5.03	-2.21	-1.47
plu0034		similarities with alternative tail fiber protein	-7.58	-2.72	-1.66
plu0947		antibiotic biosynthesis monooxygenase	-9.73	-2.86	-1.61
plu0948		transcriptional regulator (MarR family)	-7.47	-1.37	1.42
plu0965	*tcdA4*	Insecticidal toxin complex protein	-1.97	1.48	1.60
plu1846		similarities with Mg(2+) transport ATPase B	-7.16	-3.41	1.38
plu3033		hypothetical protein, similarities with unknown bacteriophage protein	-1.90	1.34	1.96
plu4185	*antJ*	transcriptional regulator	-358.06	-17.68	-1.42
plu4186	*antI*	hydrolase/peptidase	-144.45	-6.69	-1.73
plu4187	*antH*	cyclase/aromatase	-155.11	-6.39	-1.53
plu4188	*antG*	CoA ligase	-195.13	-9.75	-2.39
plu4189	*antF*	acyl carrier protein	-162.49	-9.99	-2.38
plu4190	*antE*	ketosynthase KSβ	-96.88	-7.28	-1.98
plu4191	*antD*	ketosynthase KSα	-193.06	-8.45	-2.08
plu4192	*antC*	cyclase	-161.69	-6.38	-1.55
plu4193	*antB*	PPTase	-101.93	-6.17	-1.43
plu4194	*antA*	ketoreductase	-71.71	-4.96	-1.19
plu4207		unknown	-2.55	-1.35	1.27
plu4275		similarities with Rhs-family protein of *Photorhabdus*	-8.30	-1.14	2.34
plu4603		unknown protein, zinc peptidase like domain	-1.53	-3.17	-2.39

plu0452		UDP-D-galactose:(glucosyl)lipopolysaccharide-1,6-D-galactosyltransferase	3.06	1.88	1.79
plu0939	*mltD*	membrane-bound lytic murein transglycosylase D	2.32	2.94	2.11
plu1432	*sucC*	succinyl-CoA ligase [ADP-forming] subunit beta	2.56	1.14	-1.24
plu1433	*sucD*	succinyl-CoA synthetase subunit alpha	2.08	1.01	-1.36
plu1653		ATPase AAA	3.17	-1.30	1.56
plu1895	*flhB*	flagellar biosynthesis protein FlhB	1.93	1.67	-1.60
plu1917	*flgD*	flagellar basal body rod modification protein FlgD	2.95	-1.06	-2.35
plu1920	*flgG*	flagellar basal body rod protein FlgG	1.85	-1.26	-1.82
plu1937	*fliQ*	flagellar biosynthesis protein FliQ	2.39	1.26	-1.42
plu1944	*fliJ*	flagellar biosynthesis chaperone	1.71	-1.01	-2.33
plu1947	*fliG*	flagellar motor switch protein FliG	2.46	1.11	-2.05
plu1948	*fliF*	flagellar M-ring protein FliF	1.50	-1.42	-2.47
plu2066	*prsA*	ribose-phosphate pyrophosphokinase	3.06	2.78	2.04
plu2759	*upp*	uracil phosphoribosyltransferase	3.58	2.43	1.64
plu2966		GHMP kinase	3.60	1.08	1.36
plu3071	*menB*	naphthoate synthase	2.86	-1.02	1.14
plu3072		acyl-CoA thioester hydrolase	3.72	1.29	1.38
plu3519		TonB-dependent receptor	3.40	1.67	1.65
plu4125	*frdB*	fumarate reductase iron-sulfur subunit	4.93	1.18	1.20
plu4126	*frdC*	fumarate reductase subunit C	5.06	1.06	1.25
plu4127	*frdD*	fumarate reductase subunit D	4.06	-1.02	1.39
plu4179		similar to the phenylacetyl-CoA ligase	2.86	1.37	1.37
plu4180		similarities with fatty-acyl-CoA reductase	2.94	1.43	1.36
plu4875		unknown	4.60	3.19	2.36

Table S5. Sequences of AntJ homologous used for the phylogenetic tree (Fig. 3). The 100 most similar protein sequences of AntJ were identified performing a BLASTp search against the non-redundant protein database from NCBI. Three protein sequences were removed during alignment due to the occurrence of unusual amino acids. Color code according to Fig. 3.

	Accession number	Organism
1	WP_011148289.1	*Photorhabdus luminescens*
2	WP_049584493.1	*Photorhabdus luminescens*
3	WP_046396657.1	*Photorhabdus luminescens*
4	WP_036779575.1	*Photorhabdus luminescens*
5	WP_036809535.1	*Photorhabdus luminescens*
6	WP_036840782.1	*Photorhabdus temperata*
7	WP_046975017.1	*Photorhabdus temperata*
8	WP_023044675.1	*Photorhabdus temperata*
9	WP_036844635.1	*Photorhabdus temperata*
10	WP_021325348.1	*Photorhabdus temperata*
11	WP_050110341.1	*Yersinia frederiksenii*
12	WP_050123281.1	*Yersinia frederiksenii*
13	WP_060839247.1	*Pseudomonas sp.* Os17
14	*WP_050150631.1*	*Yersinia frederiksenii]*
15	*WP_050288494.1*	*Yersinia intermedia*
16	*WP_050312930.1*	*Yersinia intermedia*
17	WP_058508488.1	*Legionella quinlivanii*
18	WP_050300205.1	*Yersinia frederiksenii*
19	WP_050072615.1	*Yersinia intermedia*
20	WP_050086694.1	*Yersinia intermedia*
21	WP_004709821.1	*Yersinia frederiksenii*
22	WP_005190475.1	*Yersinia intermedia*
23	WP_050088562.1	*Yersinia intermedia*
24	WP_050134334.1	*Yersinia frederiksenii*
25	WP_029532402.1	*Pseudomonas fuscovaginae*
26	WP_029529806.1	*Pseudomonas fuscovaginae*
27	WP_042569033.1	*Yersinia intermedia*
28	WP_055049039.1	*Devosia* sp. A16
29	KQW81476.1	*Devosia* sp. Root413D1
30	WP_029379597.1	*Pseudomonas fuscovaginae*
31	CQJ00814.1	*Yersinia frederiksenii*
32	WP_026869783.1	*Inquilinus limosus*
33	WP_050100727.1	*Yersinia frederiksenii*
34	WP_058442295.1	*Legionella brunensis*
35	WP_054207937.1	*Bosea vaviloviae*
36	WP_054428629.1	*Achromobacter* sp.
37	WP_058453455.1	*Legionella maceachernii*
38	AMG38978.1	*Achromobacter xylosoxidans*
39	*WP_054620886.1*	*Rhodocyclaceae* bacterium Paddy-1
40	WP_054473566.1	*Achromobacter* sp.
41	WP_054415390.1	*Achromobacter* sp.
42	WP_059272440.1	*Achromobacter denitrificans*
43	WP_048916042.1l	bacteria symbiont BFo1 of *Frankliniella occidentalis*

44	WP_054500924.1	*Achromobacter* sp.
45	AMH07354.1	*Achromobacter xylosoxidans*
46	WP_054516302.1	*Achromobacter* sp.
47	WP_049052979.1	*Achromobacter xylosoxidans*
48	KTL21240.1	*Achromobacter xylosoxidans*
49	AKP91749.1	*Achromobacter xylosoxidans*
50	WP_054497376.1	*Achromobacter* sp.
51	CCH07268.1	*Achromobacter xylosoxidans* NH44784-1996
52	EFV85021.1	*Achromobacter xylosoxidans* C54
53	WP_010653684.1	*Fluoribacter dumoffii*
54	AHC49505.1	*Achromobacter xylosoxidans* NBRC 15126 = ATCC 2706
55	WP_028499155.1	*Microvirgula aerodenitrificans*
56	WP_014088479.1	*Pseudogulbenkiania sp.* NH8B
57	WP_043544496.1	*Achromobacter* sp. *RTa*
58	WP_054451644.1	*Achromobacter* sp.
59	WP_028534409.1	*Paludibacterium yongneupense*
60	WP_058484690.1	*Legionella spiritensis*
61	WP_057621642.1	*Yersinia intermedia*
62	WP_052899956.1	*Erwinia iniecta*
63	IKTD24458.1	*transcriptional regulator*
64	WP_028389016.1	*Legionella fairfieldensis*
65	WP_058533473.1	*Legionella* sp. LH-SWC
66	WP_058494517.1	*Legionella drozanskii*
67	WP_027229983.1	*Phyllobacterium* sp. UNC302MFCol5.2
68	WP_043874715.1	*Legionella massiliensis*
69	WP_007873521.1	*Ochrobactrum* sp. CDB2
70	WP_058518397.1	*Legionella parisiensis*
71	WP_058480908.1	*Legionella waltersii*
72	WP_045106282.1	*Legionella hackeliae*
73	WP_025139317.1	*Achromobacter* sp. DH1f
74	WP_041598534.1	*Hahella chejuensis*
75	WP_058504736.1	*Legionella nautarum*
76	WP_045100612.1	*Tatlockia micdadei*
77	WP_028385860.1	*Legionella geestiana*
78	WP_028383162.1	*Legionella moravica*
79	WP_058477667.1	*Legionella steigerwaltii*
80	ABC28854.1	*Hahella chejuensis* KCTC 2396
81	WP_058474414.1	*Legionella quateirensis*
82	KTD77777.1	*Legionella steigerwaltii*
83	WP_058528406.1	*Legionella londiniensis*
84	WP_023492778.1	*Serratia sp.* DD3
85	IAFE61046.1	*Rahnella aquatilis* HX2
86	WP_025385387.1	*Legionella oakridgensis*
87	WP_058520358.1	*Legionella tucsonensis*
88	CZQ79410.1	*Legionella pneumophila*
89	WP_058472568.1	*Legionella quateirensis*
90	WP_058459950.1	*Fluoribacter bozemanae*
91	KEY57896.1	*Serratia* sp. DD3
92	KTD02388.1	*Legionella geestiana*
93	WP_019232362.1	*Legionella anisa*

94	WP_058450353.1	*Legionella jamestowniensis*
95	CEG60659.1	*Tatlockia micdadei*
96	WP_061468572.1	*Legionella pneumophila*
97	WP_034468061.1	*Afipia sp.* P52-10

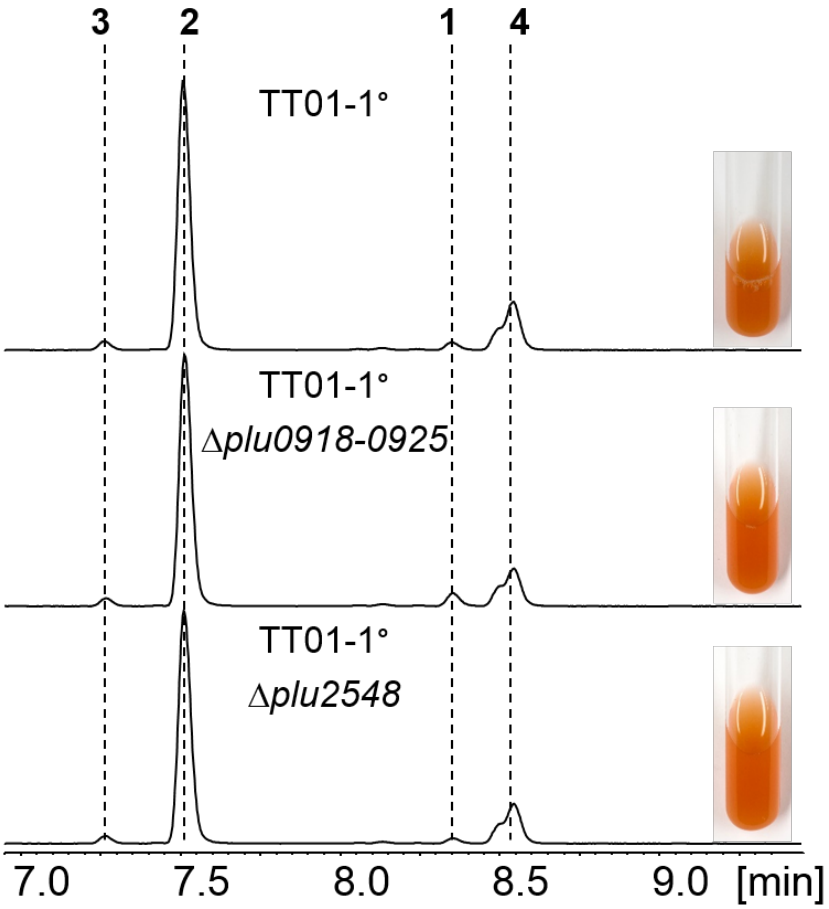

Figure S1: Deletion of *plu2548* or *plu0919* does not influence AQ (1-4) production of *P. luminescens*. Comparison of AQ production of TT01-1°, TT01-1°Δ*plu2548* and TT01-1°Δ*plu0918-plu0925*. HPLC-UV analysis (UV-chromatograms (430 nm)) of the EE extracts after cultivation for 24 h in LB broth. Plu0919 is encoded within a cluster of highly homologous PAS4-LuxR regulators [10]. For that reason the complete cluster *plu0918-plu0925* was deleted.

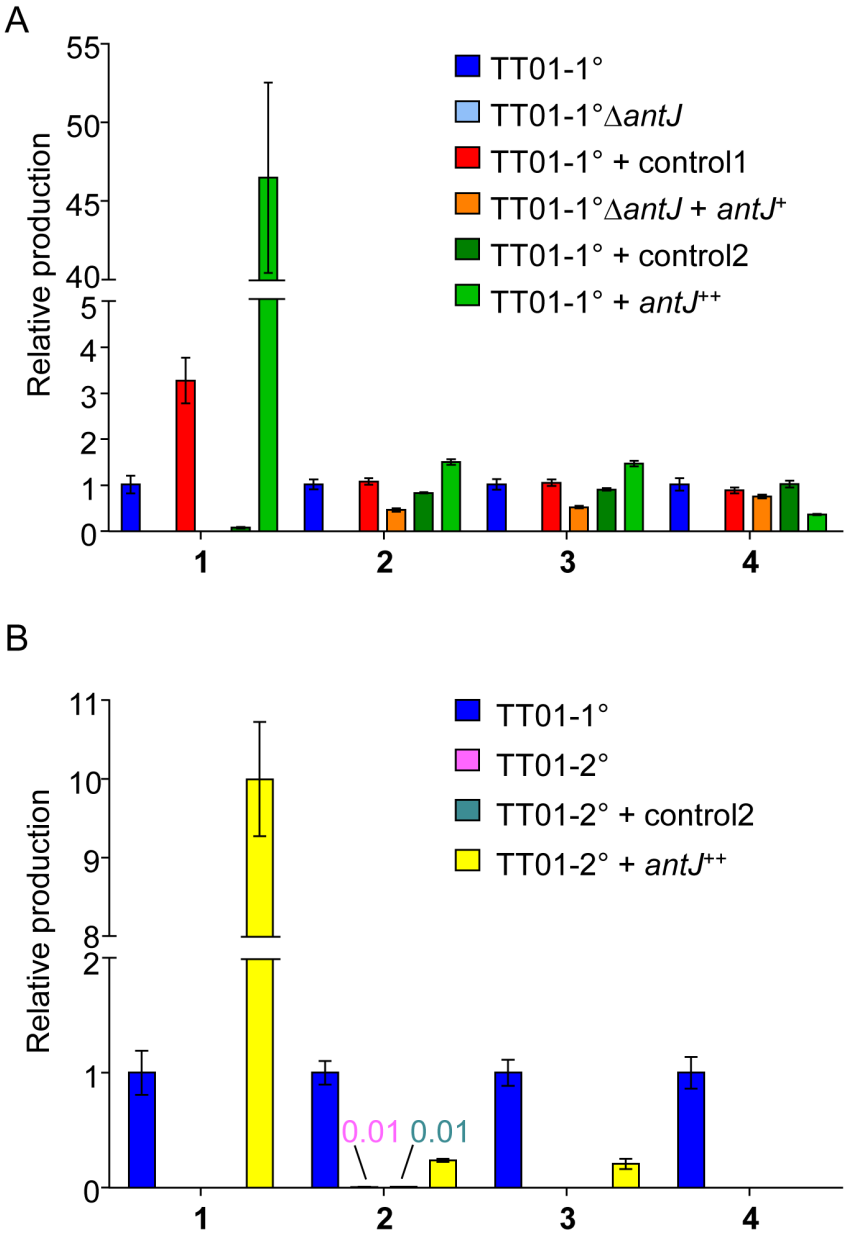

Figure S2: Quantification of AQ (1-4) production in TT01-1°, TT01-1°Δ*antJ* and TT01-2°. **A** Comparison of AQ production of TT01-1° and TT01-1°Δ*antJ* cells. **B** Comparison of AQ production of TT01-1° and TT01-2° cells. EE-extracts after 24 h of cultivation in LB broth at 30°C were measured with HPLC-UV. For AQ quantification peak areas of the HPLC-UV analysis (UV-chromatograms at 430 nm) were used. The respective peak areas were normalized with the cell density (OD_{600}) when cultures were harvested and are given relative to the production of TT01-1° of the respective compound. Extracts were prepared as triplicates. Dark blue: TT01-1°, light blue: TT01-1°Δ*antJ*, red: TT01-1° + control1 (pCOLA_tacI/l), orange: TT01-1°Δ*antJ* + *antJ*$^{+}$ (pAKH04), dark green: TT01-1° + control2 (pACYC_tacI/l), light green: TT01-1° + *antJ*$^{++}$ (pAKH05), pink: TT01-2°, petrol: TT01-2°+ control2 (pACYC_tacI/l), yellow: TT01-2°+ *antJ*$^{++}$ (pAKH05).

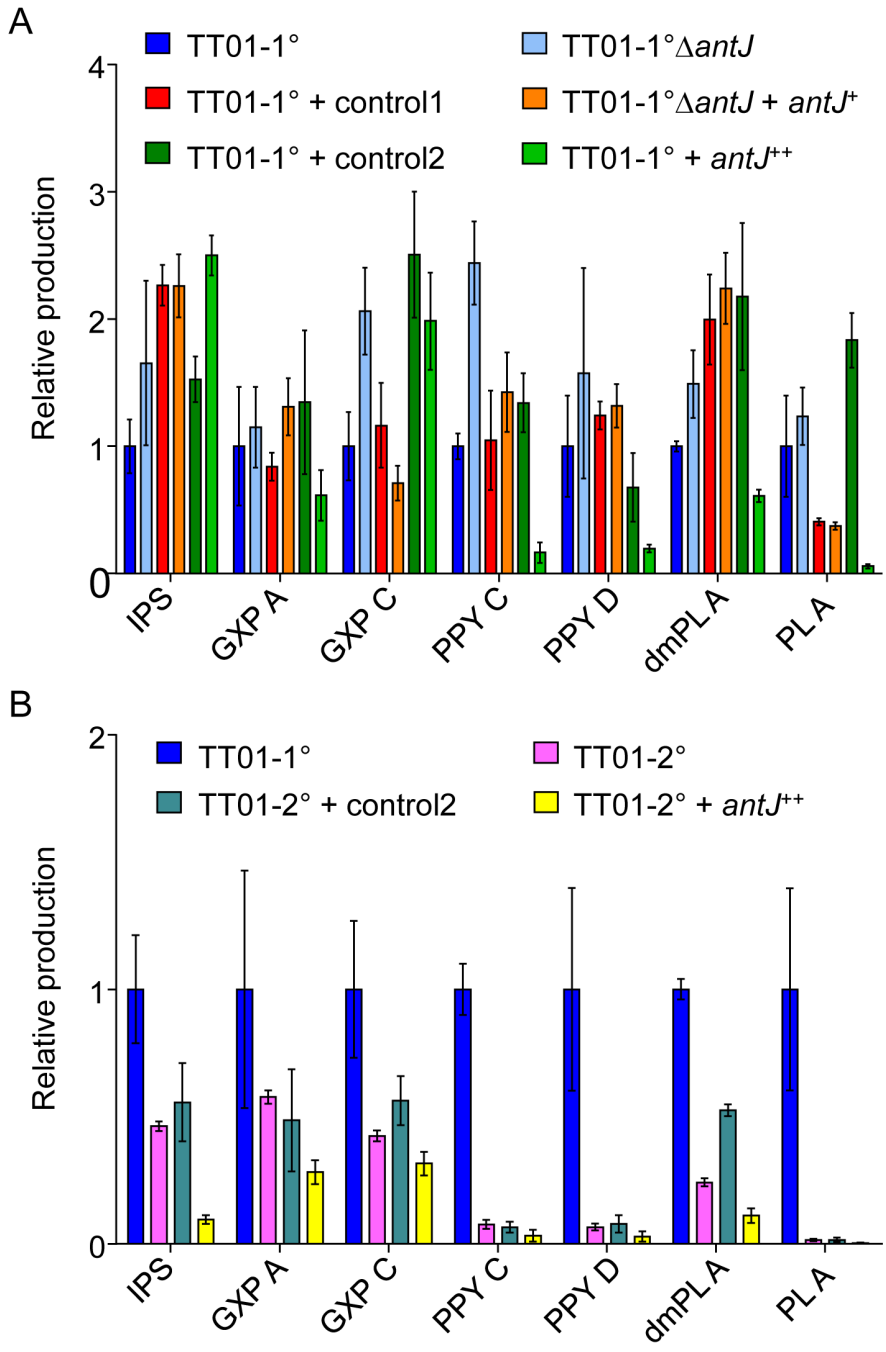

Figure S3: Quantification of secondary metabolite production in TT01-1°, TT01-1°Δ*antJ* and TT01-2°. A Comparison of secondary metabolite production of TT01-1° and TT01-1°Δ*antJ* cells. **B** Comparison of secondary metabolite production of TT01-1° and TT01-2° cells. For experimental details see Fig. S2. The secondary metabolite peak areas of the HPLC-MS analysis were determined using Bruker Compass TargetAnalysis Version 1.3. IPS: isopropylstilben; GXP A/C: GameXPeptide A/C; PPY C/D: photopyrone C/D; dmPL A: desmethyl phurealipid A; PLA A: phurealipid A. In Δ*antJ* cells PPY C and GXP C were slightly upregulated. The complemented strain Δ*antJ* + *antJ*$^{+}$ showed a very similar production compared to the corresponding wild type with the empty vector. The *antJ* overexpressing strain produced slightly more IPS but less PPYs and PLA.

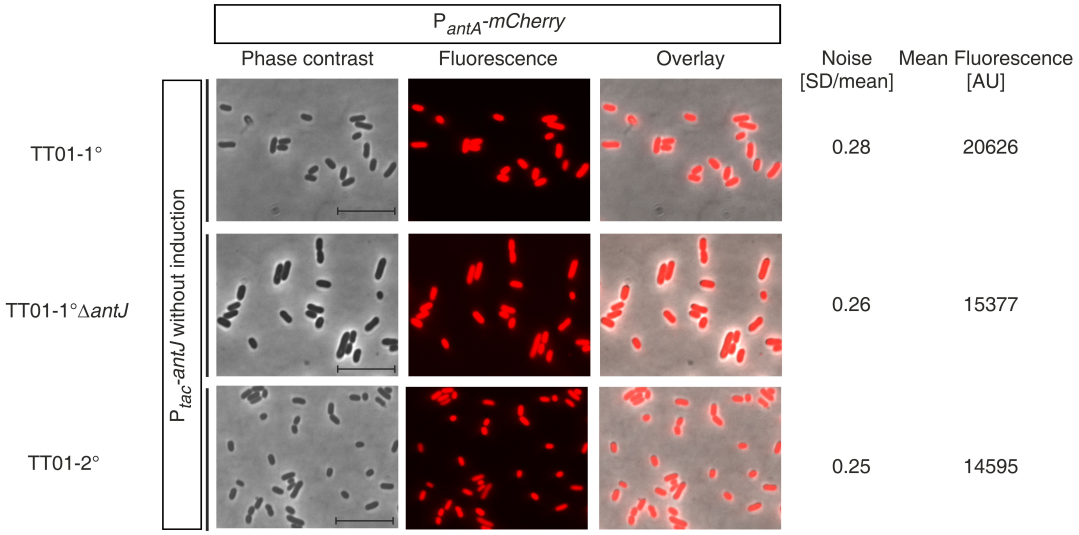

Figure S4: Single-Cell microscopy imaging of *P. luminescens* TT01-1°, TT01-1°Δ*antJ* and TT01-2° cells with chromosomally integrated P_{antA}-*mCherry* and P_{tac}-*antJ* without induction. The bars in the phase contrast pictures indicate a scale of 10 μm. The fluorescence intensities of 450 single cells were individually measured for each experiment and analyzed using the software Big Cell Brother [11]. The noise values were calculated as SD/mean from 450 cells. Representative images and values from one of three independently performed experiments are shown. AU: Arbitrary Units

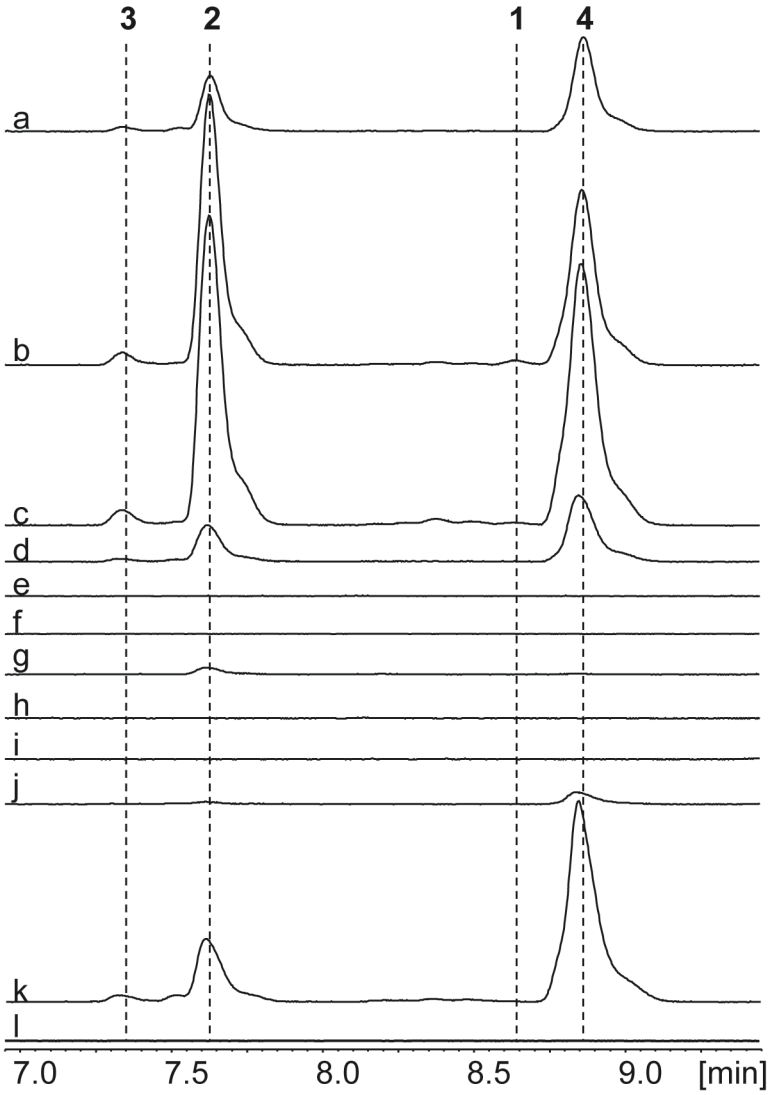

Figure S5: Comparison of AQ production of the strains for measuring the promoter activity of *antABCDEFHI* at the single cell level. HPLC-UV analysis (UV-chromatograms (430 nm)) of the EE extracts after cultivation for 24 h in LB at 30°C and 200 rpm. a: TT01-1° P_{antJ}, b: TT01-1° P_{antJ}-*antJ*, c: TT01-1° P_{tac}-*antJ*, d: TT01-1° P_{less}, e: TT01-2° P_{antJ}, f: TT01-2° P_{antJ}-*antJ*, g: TT01-2° P_{tac}-*antJ*, h: TT01-2° P_{less}, i: TT01-1°Δ*antJ* P_{antJ}, j: TT01-1°Δ*antJ* P_{antJ}-*antJ*, k: TT01-1°Δ*antJ* P_{tac}-*antJ*, l: TT01-1°Δ*antJ* P_{less}. All strains harbor P_{antA}-*mCherry* integrated at the *rpmE/glmS* site. A negative control without a promoter upstream of *mCherry* serves as negative control.

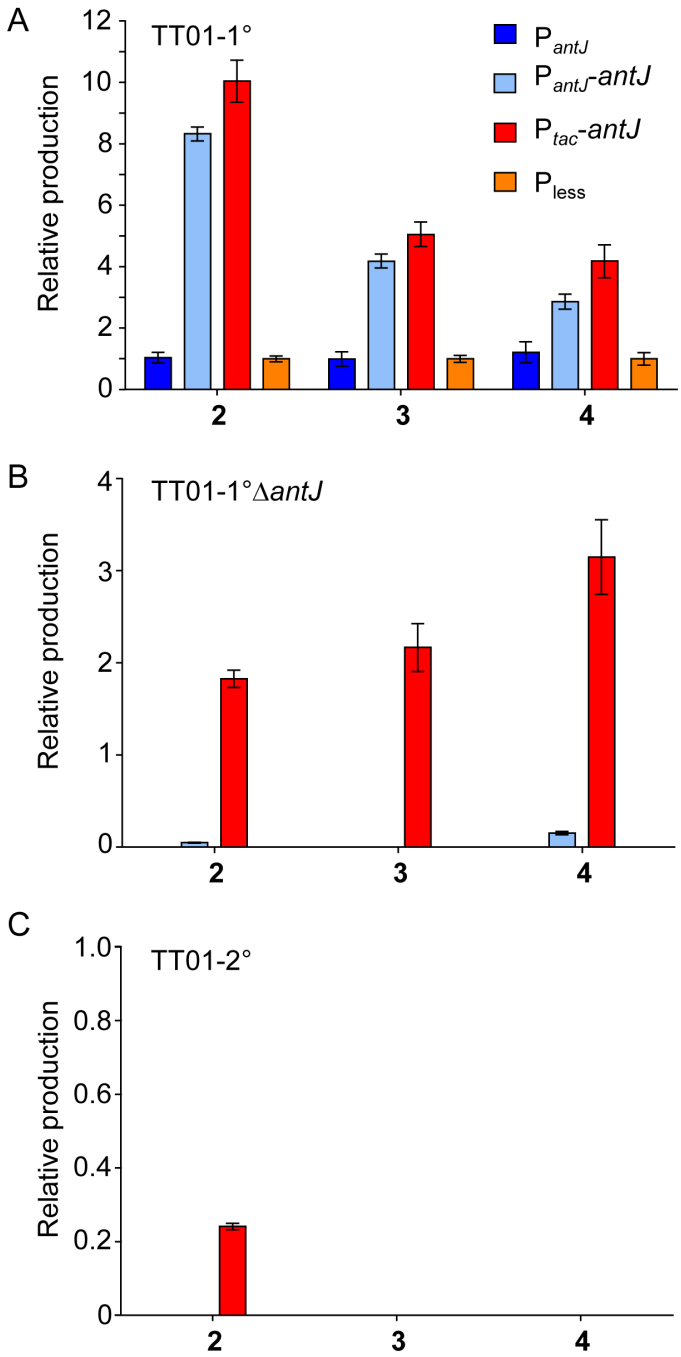

Figure S6: Quantification of the AQ production in *P. luminescens* TT01-1°, TT01-1°Δ*antJ* and TT01-2° strains with chromosomally integrated P_{antA}-*mCherry* reporter gene fusion and different levels of AntJ. A TT01-1° strains **B** TT01-1°Δ*antJ* strains **C** TT01-2° strains. EE-extracts after 24 h of cultivation in LB broth at 30°C were measured with HPLC-UV. For AQ quantification peak areas of the HPLC-UV analysis (UV-chromatograms at 430 nm) were used. The respective peak areas were normalized with the cell density (OD_{600}) when cultures were harvested and are given relative to the TT01-1° P_{less}-*mCherry* production of the respective AQ. Extracts were prepared as triplicates. All strains harbor P_{antA}-*mCherry* integrated at the *rpmE/glmS* site. A negative control without a promoter upstream of *mCherry* (P_{less}) serves as control.

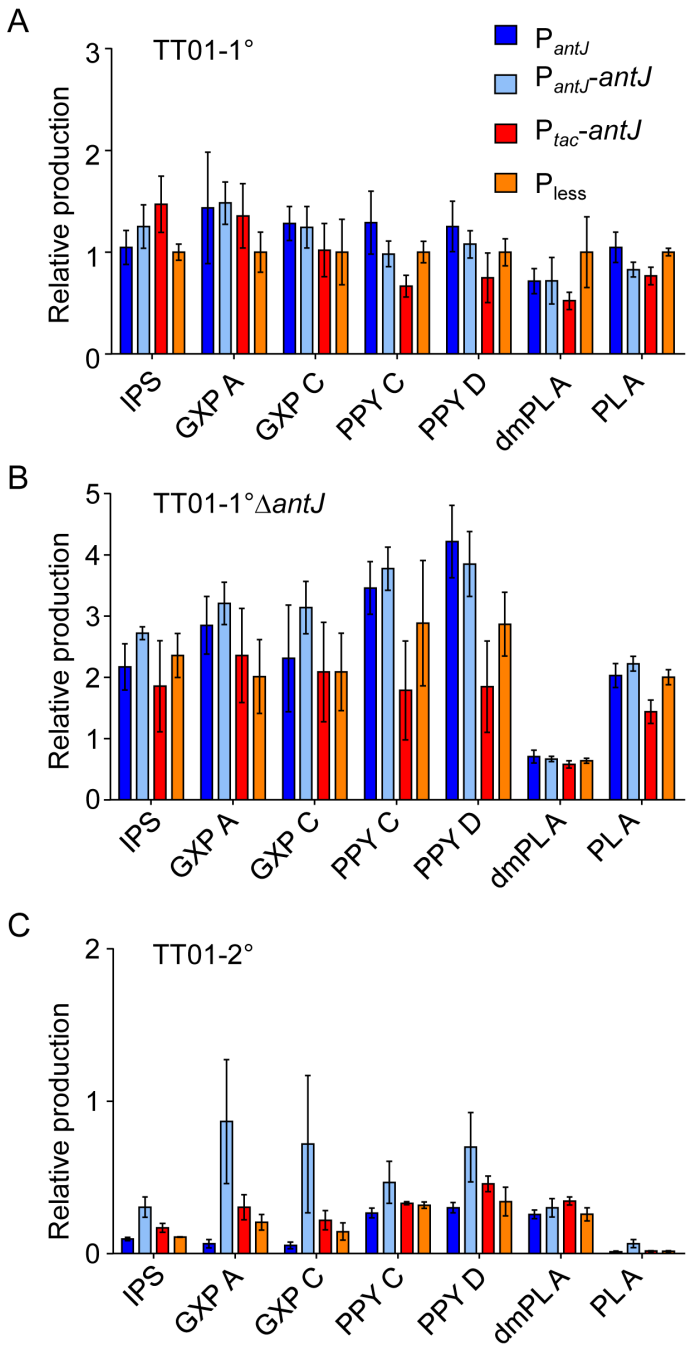

Figure S7: Quantification of the AQ production in *P. luminescens* TT01-1°, TT01-1°Δ*antJ* and TT01-2° strains with chromosomally integrated P_{antA}-*mCherry* reporter gene fusion and different levels of AntJ. A TT01-1° strains **B** TT01-1°Δ*antJ* strains **C** TT01-2° strains. For experimental details see Fig. S6. The secondary metabolites peak areas of the HPLC-MS analysis were determined using Bruker Compass TargetAnalysis Version 1.3. Secondary metabolite production is given relative to the TT01-1° P_{less}-*mCherry* production of the respective compound. IPS: isopropylstilben; GXP A/C: GameXPeptide A/C; PPY C/D: photopyrone C/D; dmPL A: desmethyl phurealipid A; PLA A: phurealipid A.

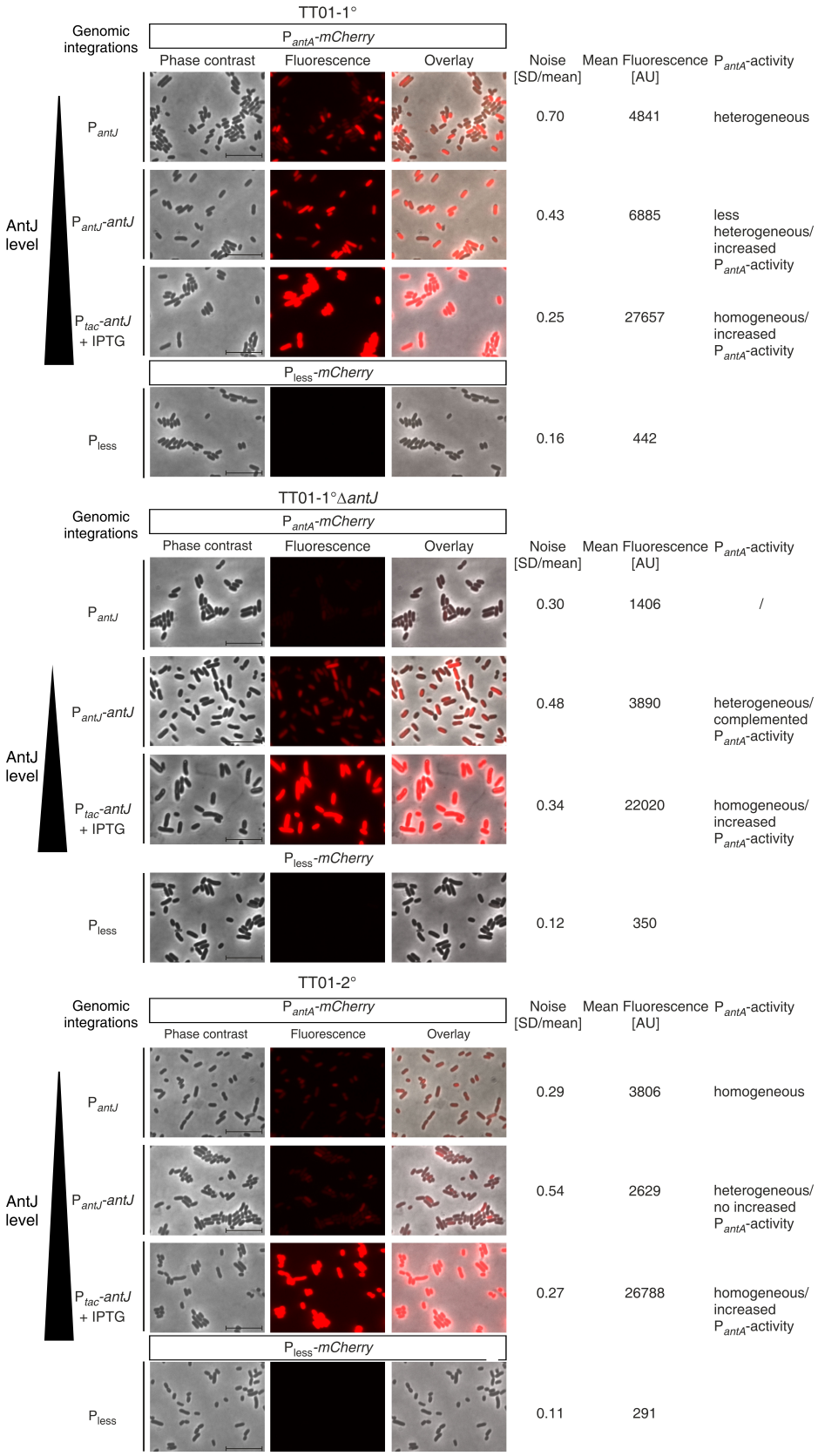

Figure S8: Single cell microscopy imaging of *P. luminescens* TT01-1°, TT01-1°Δ*antJ* and TT01-2° cells. Depicted are the pictures of the phase contrast and the fluorophore

channel as well as the respective overlay picture. The strains harbor P_{antA}-*mCherry* integrated at the *rpmE/glmS* site. A negative control without a promoter upstream of *mCherry* serves as negative control. The bars indicate a scale of 10 μm. The fluorescence intensities of 450 single cells were individually measured for each experiment and analyzed using the software Big Cell Brother [11]. The noise values were calculated as SD/mean from 450 cells. Representative images and values from one of three independently performed experiments are shown. AU= arbitrary units.

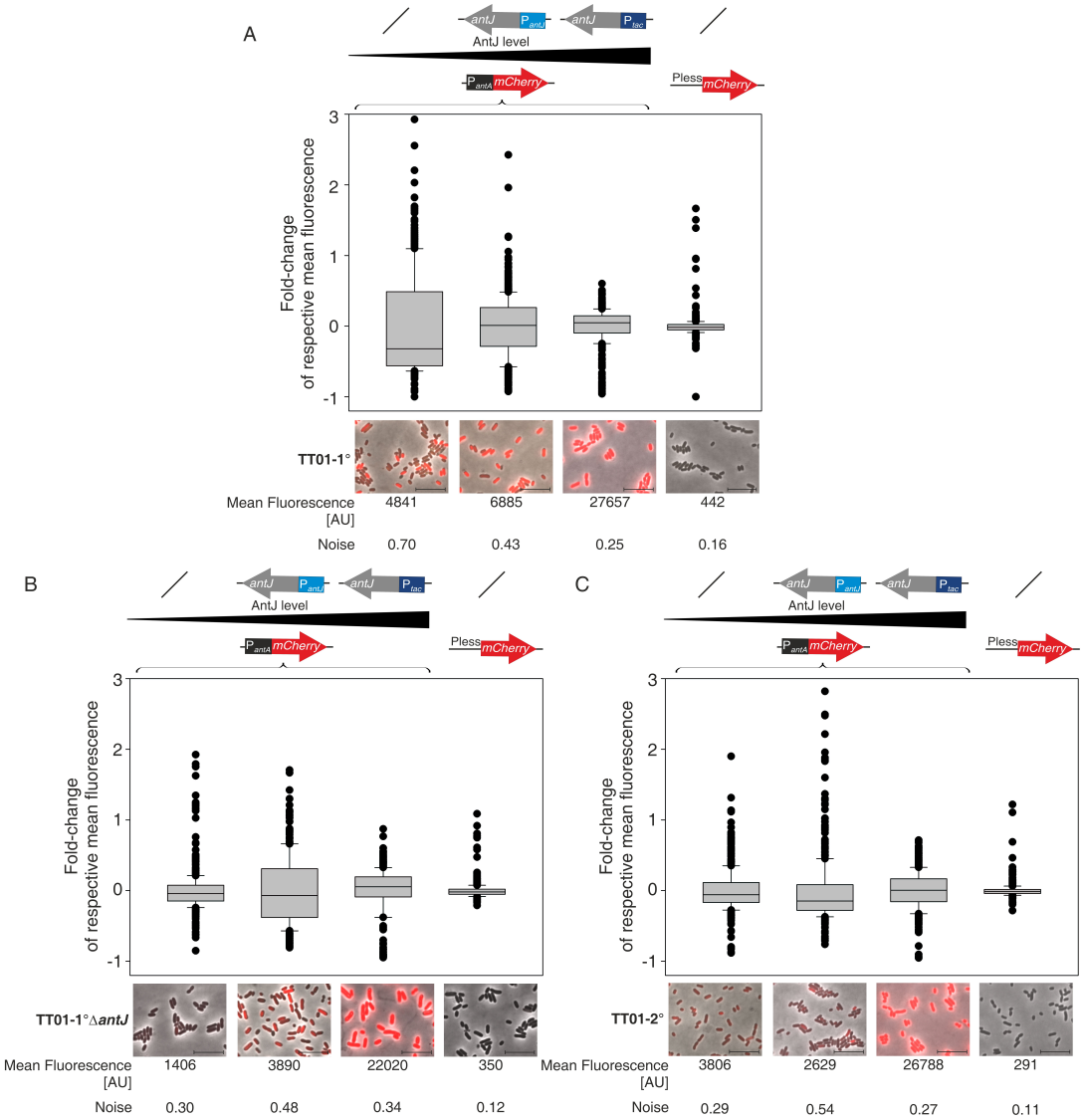

Figure S9: Degree of heterogeneity of P_{antA} activity in *P. luminescens* TT01-1°, TT01-1°*ΔantJ* and TT01-2° with different levels of AntJ. The fluorescence intensities of 450 single cells were individually measured for each experiment and analyzed using the software Big Cell Brother [11]. The noise values were calculated as SD/mean from 450 cells. The box plots represent the fold change in the mean fluorescence of the respective reporter strain. Thereby, the fluorescence value of each cell was compared to the mean fluorescence value of 450 cells of the respective reporter strain. Representative images and box plots from one of three independently performed experiments are shown. AU: arbitrary units.

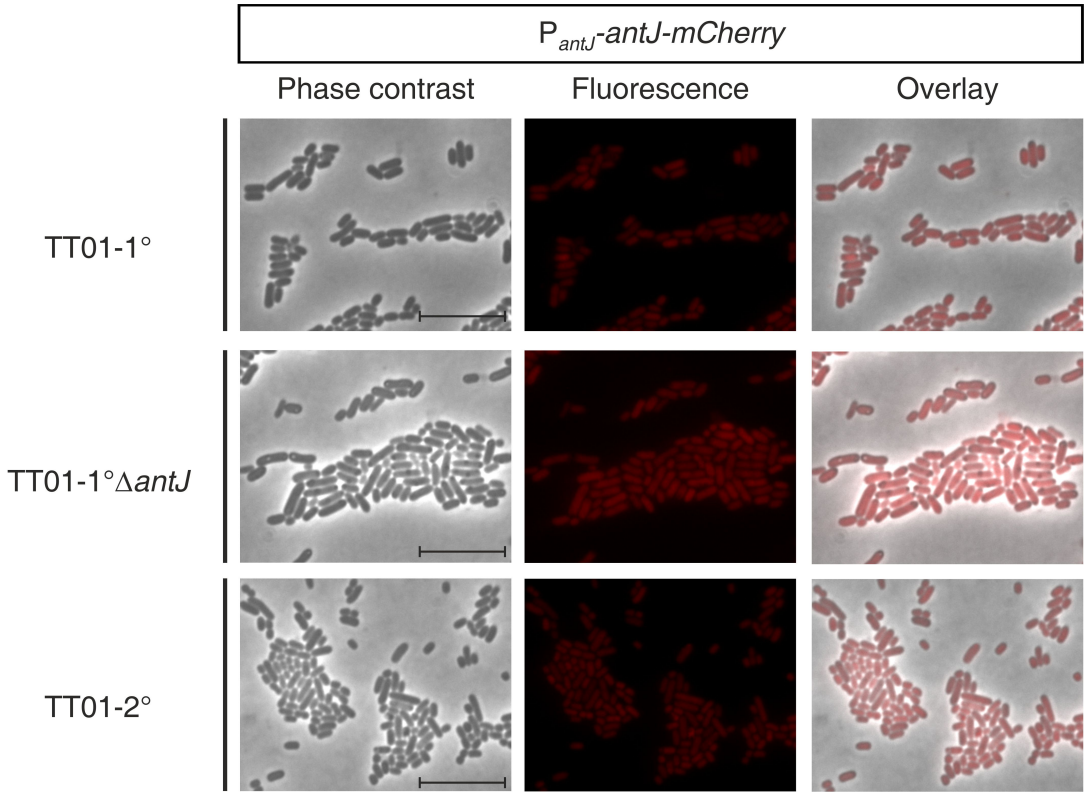

Figure S10: Single cell microscopy imaging of *P. luminescens* TT01-1°, TT01-1°Δ*antJ* and TT01-2° cells with chromosomally integrated P_{antJ}-*antJ-mCherry*. Depicted are the pictures of the phase contrast and the fluorophore channel as well as the respective overlay picture. All strains harbor P_{antJ}-*antJ-mCherry* integrated at the *rpmE/glmS* site. Representative images from one of three independently performed experiments are shown. The bars indicate a scale of 10 μm.

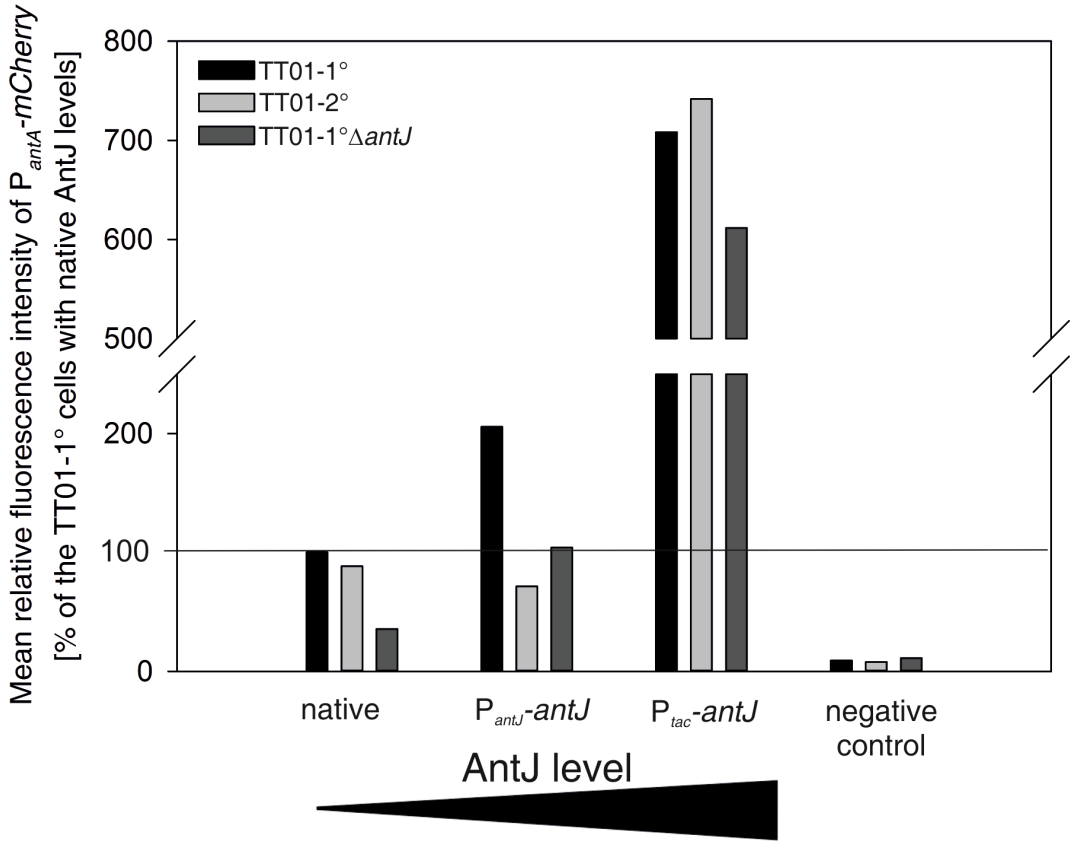

Figure S11: Influence of the AntJ levels on the mean promoter activity of *antA* in TT01-1°, TT01-2° and TT01-1°Δ*antJ* populations. The mean relative fluorescence values of P_{antA}–*mCherry* of 1350 individual cells were calculated. Then the P_{antA} activity in the TT01-1° cells under native conditions were set as 100%, marked via the horizontal black line. The mean P_{antA}-*mCherry* values of the strains TT01-1°, TT01-2° and TT01-1°Δ*antJ* with native levels of AntJ, one additional copy of *antJ* under control of its own promoter or with a strong *tac* promoter were set into relation with TT01-1° cells under native conditions given in percentage. The negative control contains a promoter-less chromosomal *mCherry* integration.

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