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Supporting Information

HexA is a versatile regulator involved in the control of phenotypic heterogeneity of *Photorhabdus luminescens*

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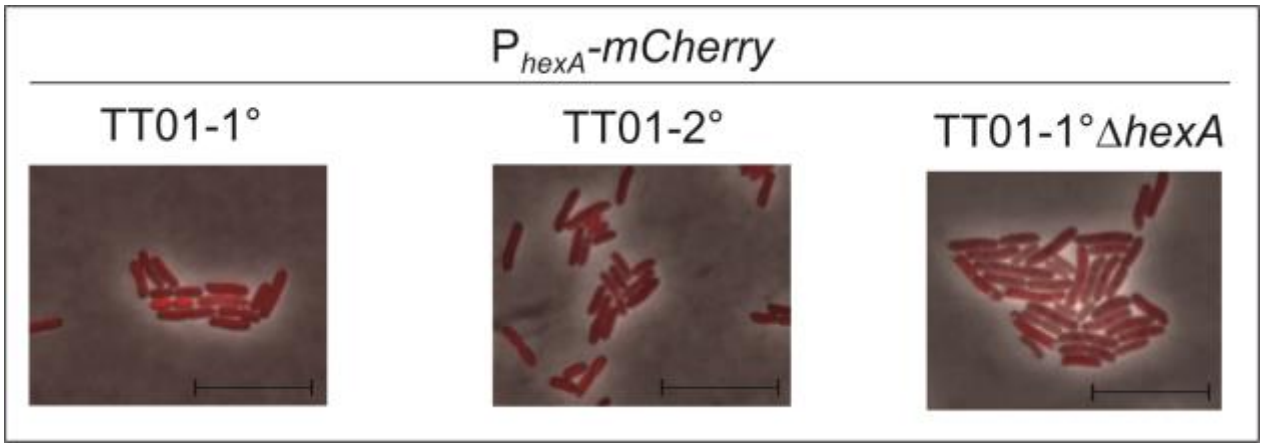


Figure A. P*hexA* activity in *P. luminescens* TT01-1°, TT01-2° and TT01-1°Δ*hexA* at the single cell level. P*hexA-mCherry* activity in TT01-1°, TT01-2° and TT01-1°Δ*hexA* after 24 h of growth. The scale depicts 10 µM. Representative images from one of three independently performed experiments are shown.

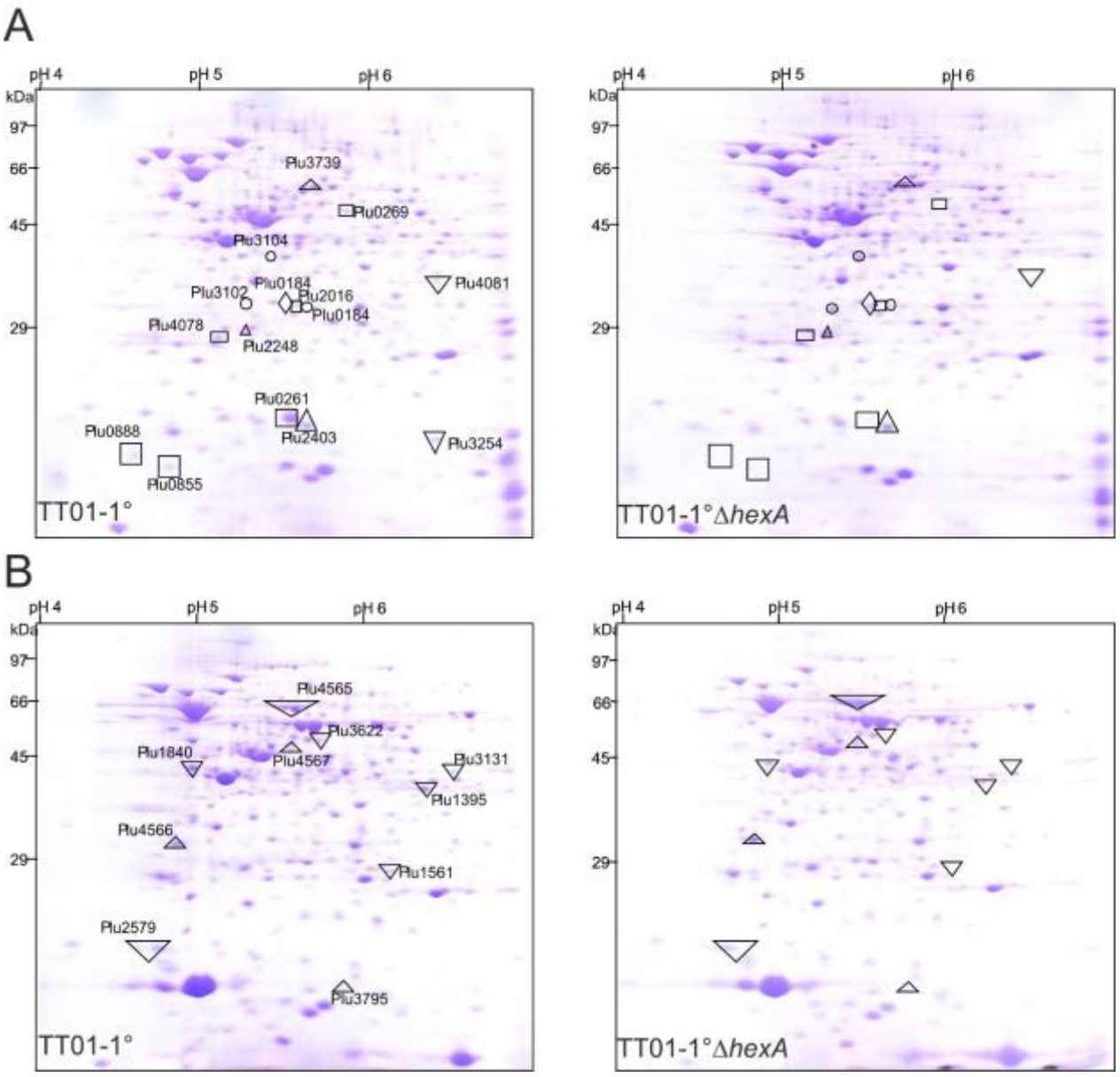


Figure B. Proteome analysis of *P. luminescens* TT01-1° and TT01-1°Δ*hexA*. Cells were cultivated and harvested in exponential (A) and in the stationary phase (B). Cytosolic proteins were extracted and then subjected to 2D-PAGE. Gels were scanned, and compared for protein spots of different sizes. Proteins with enhanced production (△), with reduced production (▽) or overproduced (◊) in the Δ*hexA* mutant and proteins that were completely absent in the Δ*hexA* mutant (□) or in the wildtype (○) were analyzed via MALDI-TOF.

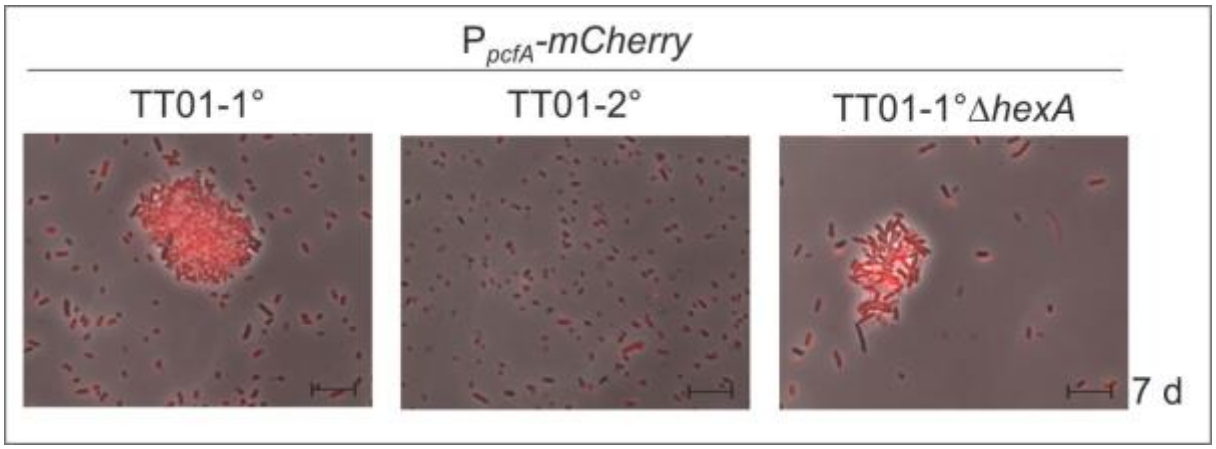


Figure C. Cell clumping in *P. luminescens* TT01-1°, TT01-2° and TT01-1°Δ*hexA* after 7 days. P_{pcfA} activity and cell clumping in TT01-1°, TT01-2° and TT01-1°Δ*hexA*. The scale depicts 10 µM. Representative images from one of three independently performed experiments are shown.

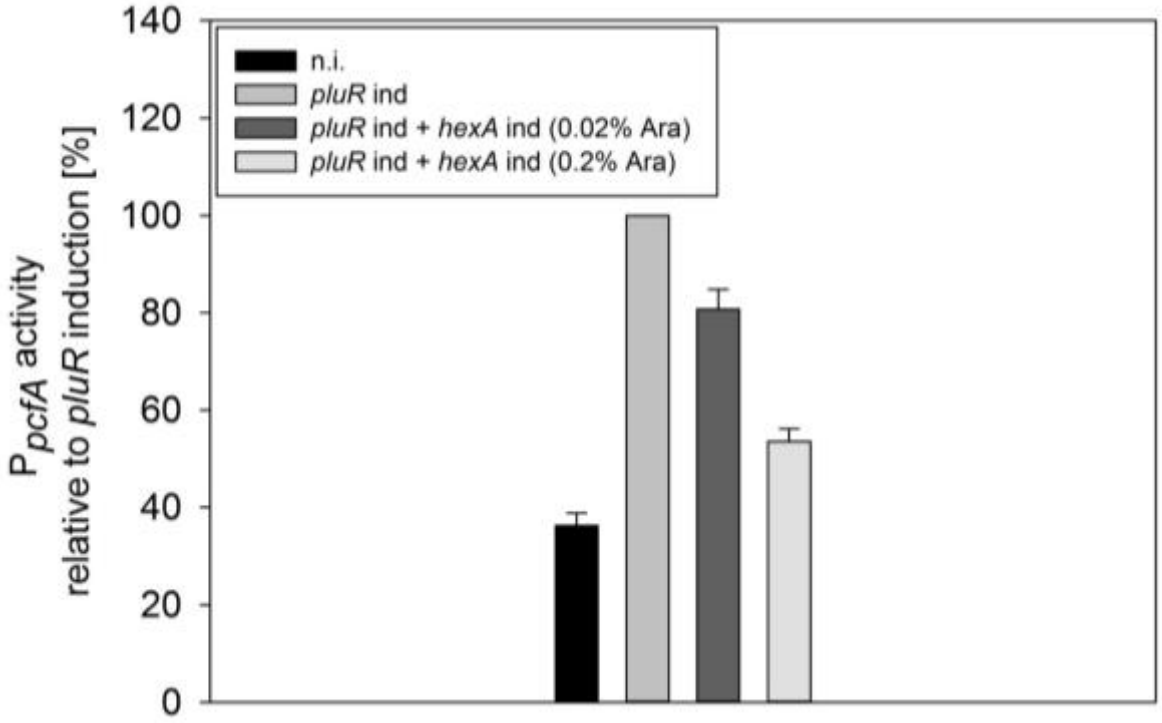


Figure D. Effect of HexA on the P_{pcfA} activity in the heterologous systems of *E. coli* Δ*lrhA*. In *E. coli* Δ*lrhA* the constructs pBAD24-P_{lac}-*pluR*_P_{ara}-*hexA* and pBBR-P_{pcfA}-*lux* were tested. The expression of *pluR* was achieved via the addition of 1 mM IPTG and *hexA* expression was induced via the addition of 0.02 and 0.2% arabinose (Ara). The figure represents three biological replicates. All values are given in percentage, relative to the maximum *pluR* induction. The values were measured as Relative Light Unit [RLU] divided by OD_{600nm}.

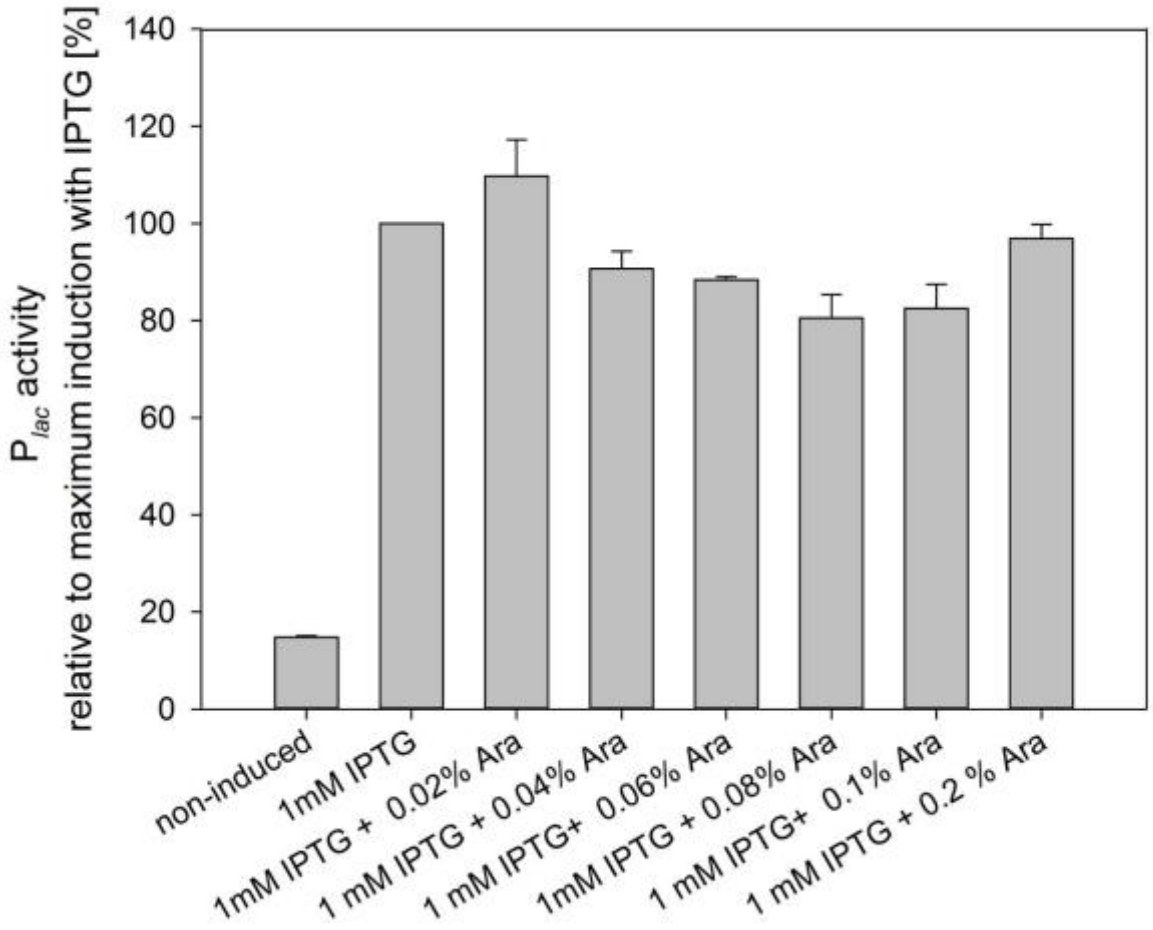


Figure E. Investigation of an effect of HexA on the *lac* promoter and the *luxCDABE* operon. The constructs pBAD24-P_{lac}-*pluR*-P_{ara}-*hexA* and pBBR-P_{lac}-*lux* were tested in *E. coli*Δ*lrhA* and 1 mM IPTG was added. Expression of *hexA* was induced via the addition of 0.02-0.2% arabinose (Ara). The graph corresponds to measurements performed 3 hours after induction. The figures represent three biological replicates. All values are expressed in percentages, relative to the values of the *pluR* maximum induction upon addition of 1 mM IPTG.

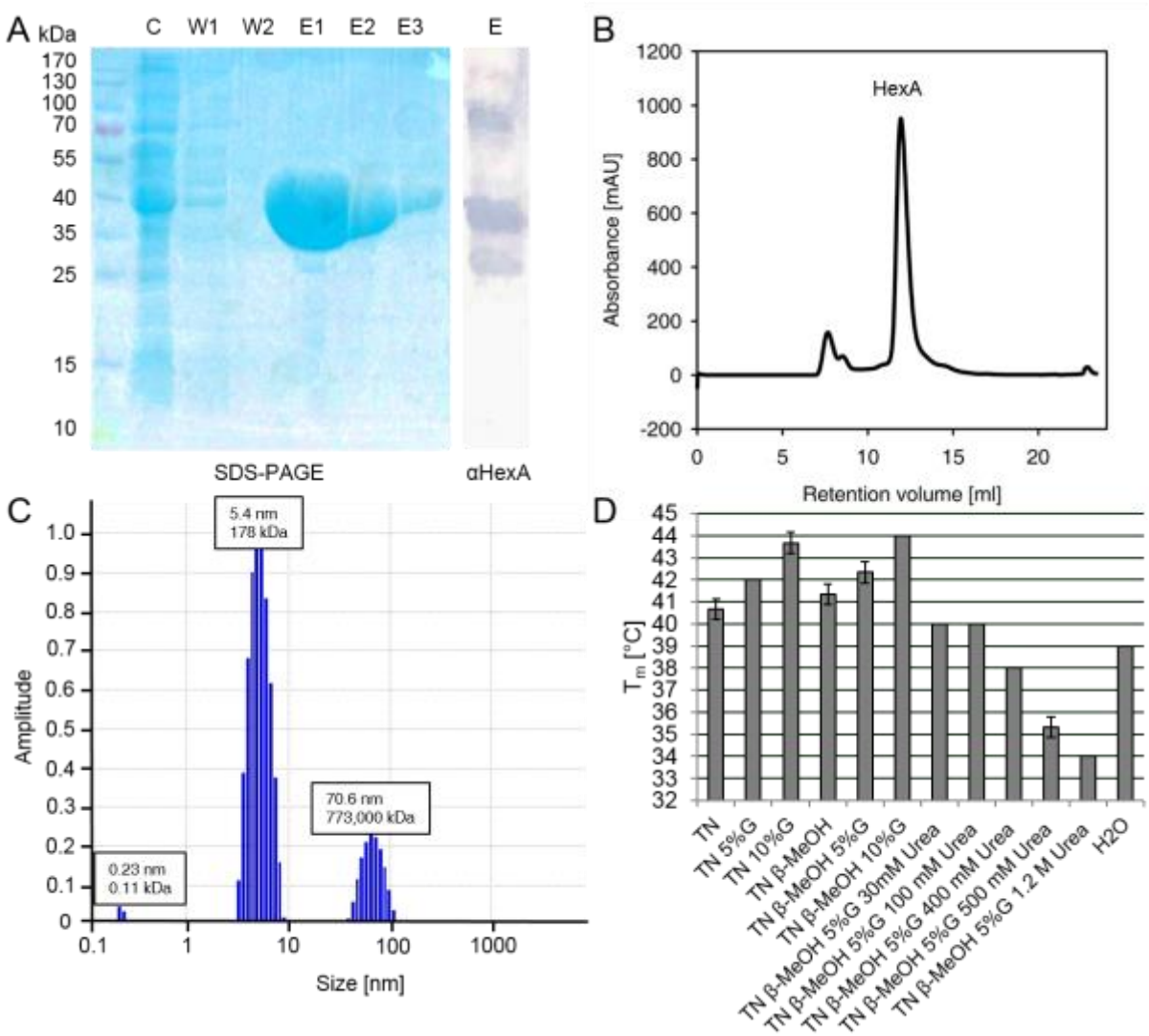


Figure F. Purification and biochemical investigation of HexA-6His. Purification of HexA via Ni-NTA affinity chromatography. Left panel shows a Coomassie blue stained SDS gel; right panel shows a Western blot with αHexA antiserum. C=cytosolic fraction; W1=washing fraction 1; W2=washing fraction 2; E1=elution fraction 1; E2=elution fraction 2; E3=elution fraction 3; E=pooled elution fraction (A). Gel filtration of purified HexA-6His (E) using Superdex 200 column (B). Size and molecular weight determination of "HexA" peak fraction (gel filtration) using Dynamic Light Scattering (DLS) (C). Stability measurement of HexA-6His in different buffers using a fluorescence-based thermal stability assay. Tm=melting temperature, TN=50mM Tris/HCl pH 7.5, 200 mM NaCl; G=glycerol; β-MeOH = 2 mM β-mercaptoethanol (D).

Table A. Bacterial Strains.

Bacterial Strain	Genotype	Reference
P. luminescens subsp. laumondi TT01-1°	Wild-type 1° variant, Rif^R	[1]
P. luminescens subsp. laumondi TT01-2°	Wild type 2° variant, Rif^R	Lab collection, Dr. David Clarke, University College Cork
P. luminescens TT01-1° Δ*hexA*	Wild-type 1° variant containing a deletion of *hexA (plu3090)*	Lab collection, Dr. David Clarke, University College Cork
P. luminescens TT01-1° P_{hexA}-*mCherry*	TT01-1° harboring P_{hexA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	[2]
P. luminescens TT01-2° P_{hexA}-*mCherry*	TT01-2° harboring P_{hexA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° Δ*hexA* P_{hexA}-*mCherry*	TT01-1° Δ*hexA* harboring P_{hexA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° P_{hexA}-*hexA*-*mCherry*	TT01-1° harboring P_{hexA}-*hexA*-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-2° P_{hexA}-*hexA*-*mCherry*	TT01-2° harboring P_{hexA}-*hexA*-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° Δ*hexA* P_{hexA}-*hexA*-*mCherry*	TT01-1° Δ*hexA* harboring P_{hexA}-*hexA*-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° P_{luxC}-*mCherry*	TT01-1° harboring P_{luxC}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	[2]
P. luminescens TT01-2° P_{luxC}-*mCherry*	TT01-2° harboring P_{luxC}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study

P. luminescens TT01-1° Δ*hexA* P_{luxC}-*mCherry*	TT01-1° Δ*hexA* harboring P_{luxC}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° P_{hfq}-*mCherry*	TT01-1° harboring P_{hfq}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-2° P_{hfq}-*mCherry*	TT01-2° harboring P_{hfq}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° Δ*hexA* P_{hfq}-*mCherry*	TT01-1° Δ*hexA* harboring P_{hfq}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° P_{pcfA}-*mCherry*	TT01-1° harboring P_{pcfA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-2° P_{pcfA}-*mCherry*	TT01-2° harboring P_{pcfA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
P. luminescens TT01-1° Δ*hexA* P_{pcfA}-*mCherry*	TT01-1° *ΔhexA* harboring P_{pcfA}-*mCherry* reporter integrated at the *rpmE*/*glmS* site, Kan^R, $Gent^R$	This study
E. coli Dh5α λ*pir*	*recA1, gyrA (lacIZYA-argF) (80d lac [lacZ] M15) pir RK6*	[3]
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* ∆*hemA*	[4]
E. coli BL21 (DE3) Star	F^- *omp*T *hsd*S_B (r_B^- m_B^-) *gal dcm rne*131 (DE3)	Invitrogen
E. coli JW2284	Kan^R, BW25113 *lrhA::npt*	[5]
E. coli ΔlrhA	Removal of the *npt* cassette in *E. coli* JW2284 by P1 transduction	Dr. Sophie Brameyer, unpublished
Sh. oneidensis MR1 S79	Wild type isolate	[6]

Table B. Plasmids.

Plasmid	Genotype	Reference
pPINT-*mCherry*	Km^R, Gm^R and *mCherry* in pPINT	[2]
pPINT-P_{hexA}-*mCherry*	Km^R, Gm^R, *hexA* (*plu3090*) promoter upstream of *mCherry*	[2]
pPINT-P_{hexA}-*hexA-mCherry*	Km^R, Gm^R, *hexA* promoter upstream of *hexA (plu3090)-mCherry*	This study
pPINT-P_{luxC}-*mCherry*	Km^R, Gm^R, *luxC (plu2079)* promoter upstream of *mCherry*	[2]
pPINT-P_{hfq}-*mCherry*	Km^R, Gm^R, *hfq (plu4581)* promoter upstream of *mCherry*	This study
pPINT-P_{pcfA}-*mCherry*	Km^R, Gm^R, *pcfA (plu4568)* promoter upstream of *mCherry*	This study
pBAD24-*pluR*	Ap^R, *pluR* (*plu4562*) in pBAD24	[7]
pBAD24-*yehU*	Ap^R, *yehU*-6His in pBAD24 with a C-terminal HisTag	[8]
pBAD24-*hexA*	Ap^R, *hexA*-6His (*plu3090*) in pBAD24 with a C-terminal HisTag	This study
pCOLA-*ppyS*-His-*pluR*	Km^R, *ppyS (plu4844*) and 6His-*pluR* (*plu4562*) in pCOLA, IPTG inducible	Dr. Sophie Brameyer, unpublished
pBAD24-P_{ara}-*pluR*_P_{lac}-*hexA*	Ap^R, *pluR* (*plu4562*) under the control of an arabinose inducible promoter, *hexA* (*plu3090*) under the control of an IPTG inducible promoter	This study
pBAD24-P_{lac}-*pluR*_P_{ara}-*hexA*	Ap^R, *pluR* (*plu4562*) under the control of an IPTG inducible promoter, *hexA*	This study

	(*plu3090*) under control of an arabinose inducible promoter	
pBBR1-P$_{pcfA}$-*lux*	Gm^R, l*uxCDABE* under the control of the *pcfA* (*plu4568*) promoter	[7]
pBBR1-P$_{pcfA}$-s1-*lux*	Gm^R, *luxCDABE* under the control of the truncated promoter construct P$_{pcfA}$-S1	Dr. Sophie Brameyer, unpublished
pBBR-P$_{pcfA}$-s2-*lux*	Gm^R, *luxCDABE* under the control of the truncated promoter construct P$_{pcfA}$-S2	Dr. Sophie Brameyer, unpublished
pBBR-P$_{lac}$-*lux*	Gm^R, *luxCDABE* under the control of the *lac* promoter	This study
pACYC-Duet1	Cm^R, Expression vector, IPTG inducible	Novagen®
pACYC-*hexA*	Cm^R, *hexA* (*plu3090*) in pACYC-Duet1	This study
pACYC-P$_{lac}$-*hexA*_P$_{ara}$-*pluR*	Cm^R, *pluR* (*plu4562*) under the control of an arabinose inducible promoter, *hexA* (*plu3090*) under the control of an IPTG inducible promoter	This study
pEYFP	Ap^R, *lac*-promoter upstream of *eYFP*	Takara-Clonetech, Saint-Germain-en-Laye, France)
pD132	Cm^R, ori R6K, oriT RK2, *sacB*	[9]
pDS-*hexA*	Flanking regions of *hexA* (*plu3090*) in pD132	This study

Table C. Oligonucleotides.

Primer name	Sequence (5`-3`)
PhexA-BamHI_fwd	GCTGGATCCTCTTACCTTATCTTGGTAAA
hexA-XmaI _rev	GCTCCCGGGCTCATCAATAATATCGTCATCATCA
Phfq-NheI_fwd	GCGGCTAGCTCACTGAACTGACTACATTG
Phfq-BamHI_rev	GCTGGATCCTCTATATTTTCCTTATTTTGTT
PpcfA-NheI_fwd	AATGGAGCTAGCAGCAGAATTCGGGTTAGTTATCTATGC
PpcfA-XmaI_rev	ACTAAGCCCGGGACCAGCTTTATCCCTTATGTC
check-mcherry_ins_fwd	CTGGTTTCATAATTTCGCC
check-mcherry-ins_rev	GGCCTTCCTTCTCCTTCAC
check-rpmE_fwd	CTCCCAAATAAAGTTTAGG
check-glmS_rev	GTACGTGAATCTGATTTTG
oriT_fwd	CAGGGTTATGCAGCGGAAA
gmRpNPTS_fwd	GATAAGCTGTCAAACATGAGAGTAGCGTATGCGCTCAC
Plac(h)_fwd	ATTGCATTTATCATGGTATATCTCCTTATTAAA
PlacI-SalI_rev	GCTGTCGACTCACTGCCCGCTTTCCAGTC
hexA_fwd	ATGATAAATGCAAATCGTC
hexA-PstI-rev	GCTCTGCAGTTACTCATCAATAATATCG
pBAD24_seq_fwd	GCCGTCACTGCGTCTTTTACTGG
pBAD24_seq_rev	CGCTACGGCGTTTCACTTCTG
hexA-EcoRI_fwd	GCTGAATTCATGATAAATGCAAATCGTCC
hexA-NdeI_rev	GCGCATATGCTCATCAATAATATCGTCATCATC
Plac-PluR_fwd	TCTTCAAAGCTTGCGGCCGCATAATG
PluR-PstI_rev	GCGCTGCAGGTTATATGATTAGATTATATGCTATTGC
lacI_fwd	CAAGCTTTGAAGATCGAATGGCGCAAAACCTT
lacI-SalI_rev	GCTGTCGACTCACTGCCCGCTTTCCAGTC

check-PlachexA_fwd	CTACCAGAGAAGTTGAAGT
hexA-NcoI_fwd	GCTCCATGGATGATAAATGCAAATCGTCC
hexA-SalI_rev	GCGGTCGACTTACTCATCAATAATATC
check-pACYC_fwd	ATTCACCACCCTGAATTGA
check-pACYC_rev	CTAGTTATTGCTCAGCGGT
araCPluR_fwd	GCGCATATGACTCCGTCAAGCCGTCAA
pluR-XhoI_rev	TAGCCCTCGAGCTGTGATGATGATGATGATGATGATGATG
	ACGACCTTCGATATGGCCGCTTATATGATTAGATTATATGC
PpcfA-Btn_fwd	TATTTGTCTTTATAATGATAAT
PpcfA_rev	ACCAGCTTTATCCCTTATGTC
sacB-Btn_fwd	GCAGAAGTTTTTGACTTTCTTG
sacB_rev	ACATCTGACGGAAAAATCCGT
Plac-NheI_fwd	GCGGCTAGCGCGCAACGCAATTAATGTG
Plac-BamHI_rev	CGCGGATCCAGCTGTTTCCTGTGTGAAA
check-pBBR-Plac_fwd	CCGTCGTATTAAAGAGGGG
FA_hexA_fwd	GAATTGTTGTTGTTTTTTA
FA_hexA_rev	CATTGTTTATTCATCACTTT
FB_hexA_fwd	TAATATCTGAAACACTTCTC
FB_hexA_rev	AATCAATGATTGATGGAGTG

Table D. Proteins with altered production in the proteome of TT01-1°Δ*hexA* compared to TT01-1°. Differences in the cytosolic proteome were detected in the exponential (EX) and stationary (STAT) growth phase.

Protein	Putative function	Growth phase	Δ*hexA*/wild-type
Plu0184 (CpmC)	Role in Carbapenem biosynthesis		+4.2
Plu0261	Similiarities with type 1 fimbrial protein precursor	EX	n.d. in Δ*hexA*
Plu0269	Unknown, hypothetical secreted protein	EX	n.d. in Δ*hexA*
Plu0885	Pyocin S3 protein, „killer protein“	EX	n.d. in Δ*hexA*
Plu0888	Colicin/Pyocin protein, „killer protein“	EX	n.d. in Δ*hexA*
Plu1395	Cystein Synthase A	STAT	-1.7
Plu1561	Ca^{2+}-dependent cell adhesion molecule	STAT	-2.6
Plu1840	unknown	STAT	-1.6
Plu2016	PAS4-LuxR regulator	EX	n.d. in Δ*hexA*
Plu2248	Carbonic anhydrase	EX	+4.0
Plu3102	methyltransferase	EX	n.d. in WT
Plu3104	unknown	EX	n.d. in WT
Plu3110 (ArgM)	Succinylornithine transaminase	STAT	-1.4
Plu3254	Hcp family T6SS protein CtsH1	EX	-2.7
Plu3622 (AceF)	dihydrolipoamide acetyltransferase; pyruvate dehydrogenase subunit E2	STAT	-2.2
Plu3739 (AldB)	Aldehyde Dehydrogenase B	EXP	+1.8
Plu3795	unknown	STAT	+2.4
Plu4078	Dimethylmenaquinone methyltransferase	EXP	n.d. in Δ*hexA*
Plu4081	Putative aldolase		-3.2
Plu4565	Cysteine synthase	STAT	+2.0

(PcfA)			
Plu4567	Ariginosuccinate synthase	STAT	+2.0
(PcfB)			
Plu4566	Glycine amidino transferase	STAT	+2.1
(PcfC)			

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