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Authors	Langer, Angela;Moldovan, Adriana;Harmath, Christian;Joyce, Susan A.;Clarke, David J.;Heermann, Ralf
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Supporting Information

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

Angela Langer¹, Adriana Moldovan¹, Christian Harmath¹, Susan A. Joyce², David J. Clarke² and Ralf Heermann^{1*}

¹*Bereich Mikrobiologie, Biozentrum Martinsried, Ludwig-Maximilians-Universität München, München, Germany*

²*School of Microbiology and Microbiome Institute, University College Cork, Cork, Ireland*

*Corresponding author
heermann@lmu.de

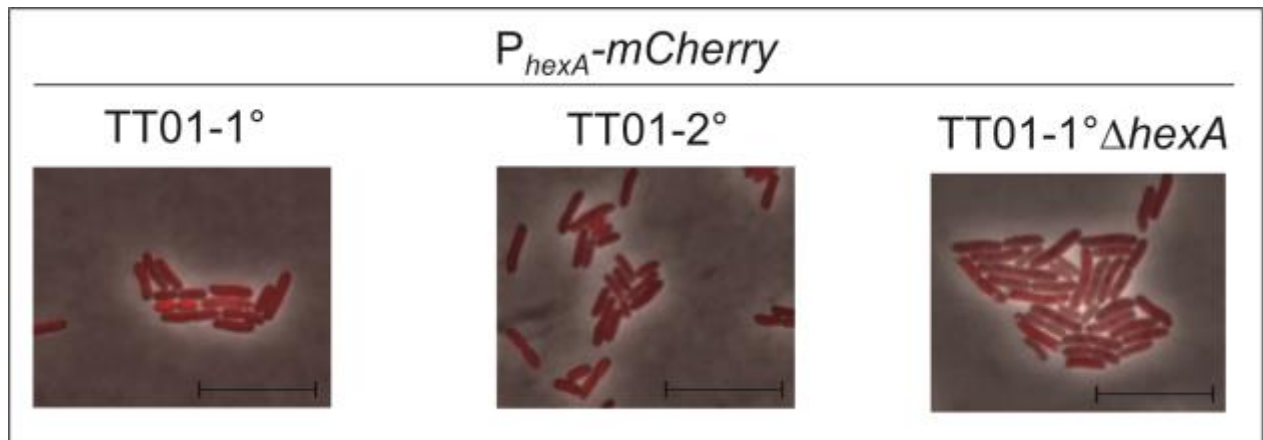


Figure A. P_{hexA} activity in *P. luminescens* TT01-1°, TT01-2° and TT01-1°ΔhexA at the single cell level. P_{hexA} -mCherry activity in TT01-1°, TT01-2° and TT01-1°ΔhexA after 24 h of growth. The scale depicts 10 μM. Representative images from one of three independently performed experiments are shown.

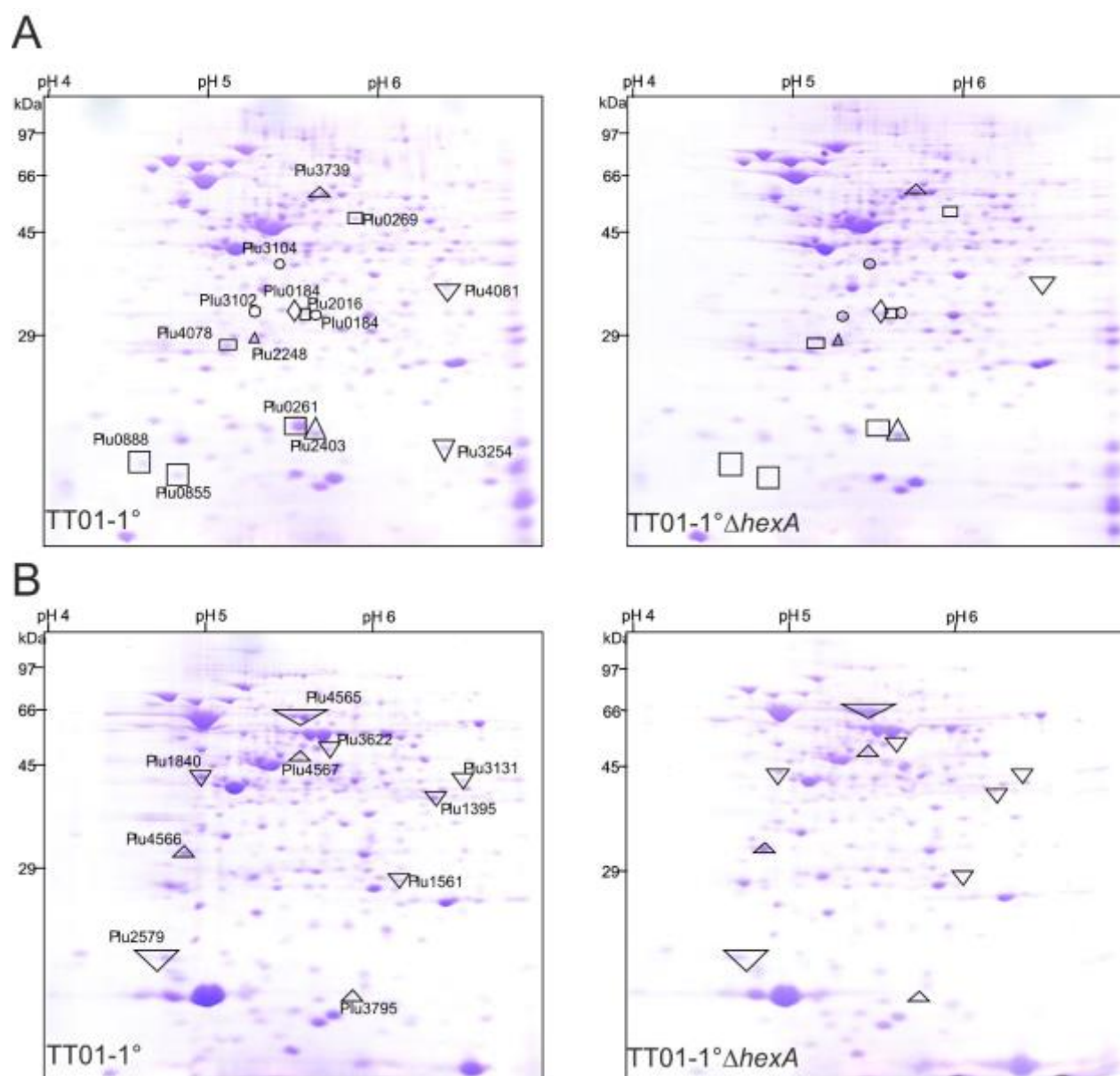


Figure B. Proteome analysis of *P. luminescens* TT01-1° and TT01-1° $\Delta hexA$. Cells were cultivated and harvested in exponential (A) and in the stationary phase (B). Cytosolic proteins were extracted and then subjected to 2D-PAGE. Gels were scanned, and compared for protein spots of different sizes.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

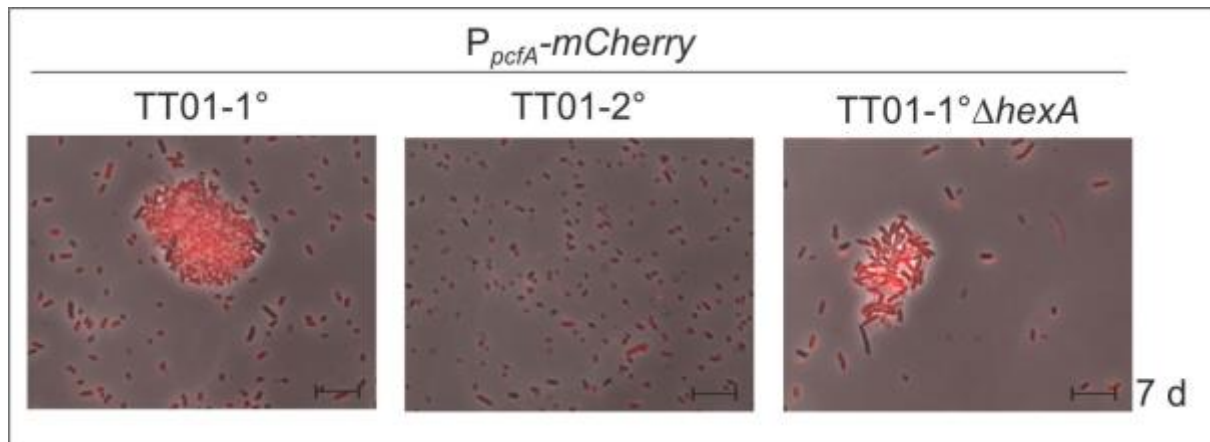


Figure C. Cell clumping in *P. luminescens* TT01-1°, TT01-2° and TT01-1°ΔhexA after 7 days. P_{pcfA} activity and cell clumping in TT01-1°, TT01-2° and TT01-1°ΔhexA. The scale depicts 10 μ M. Representative images from one of three independently performed experiments are shown.

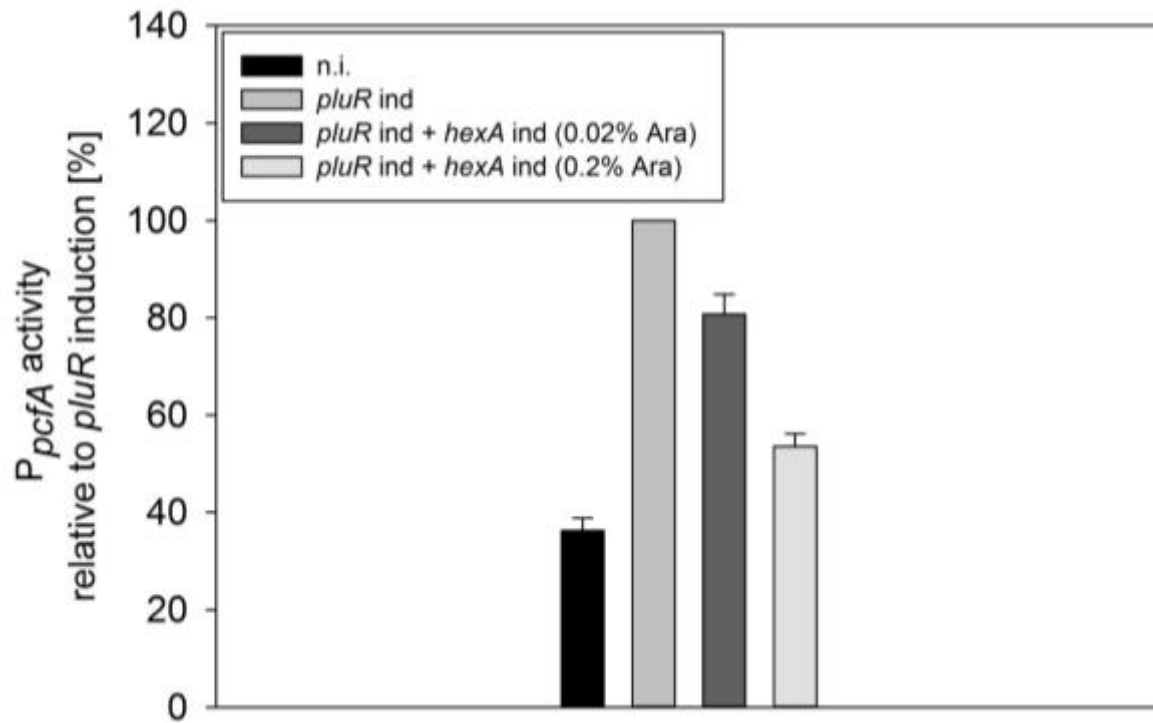


Figure D. Effect of HexA on the P_{pcfA} activity in the heterologous systems of *E. coli* $\Delta l rhA$.

In *E. coli* $\Delta l rhA$ 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} .

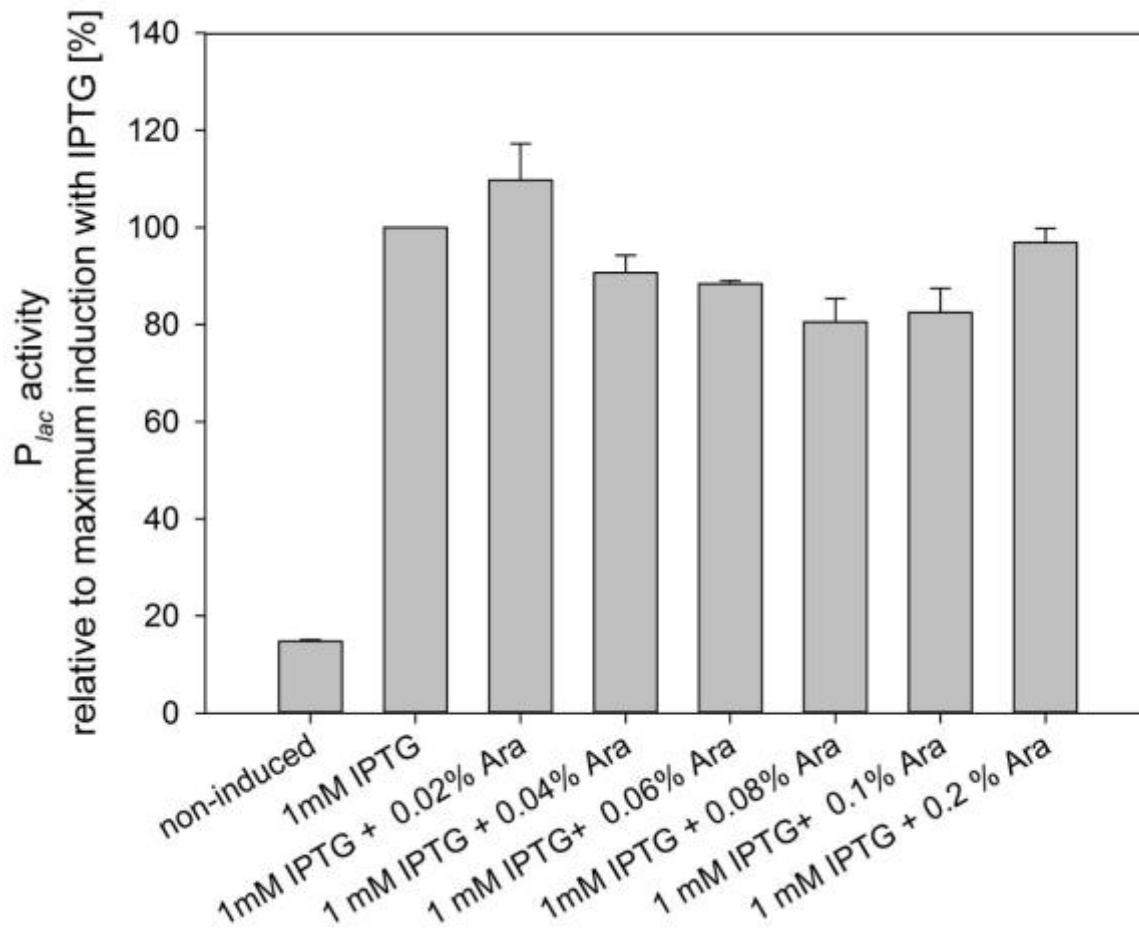


Figure E. Investigation of an effect of HexA on the *lac* promoter and the *luxCDABE* operon. The constructs pBAD24- P_{lac} -*pluR*- P_{ara} -*hexA* and pBBR- P_{lac} -*lux* were tested in *E. coli* Δ *lrhA* and 1 mM IPTG was added. Expression of *hexA* was induced via the addition of 0.02-0.2% arabinose (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.

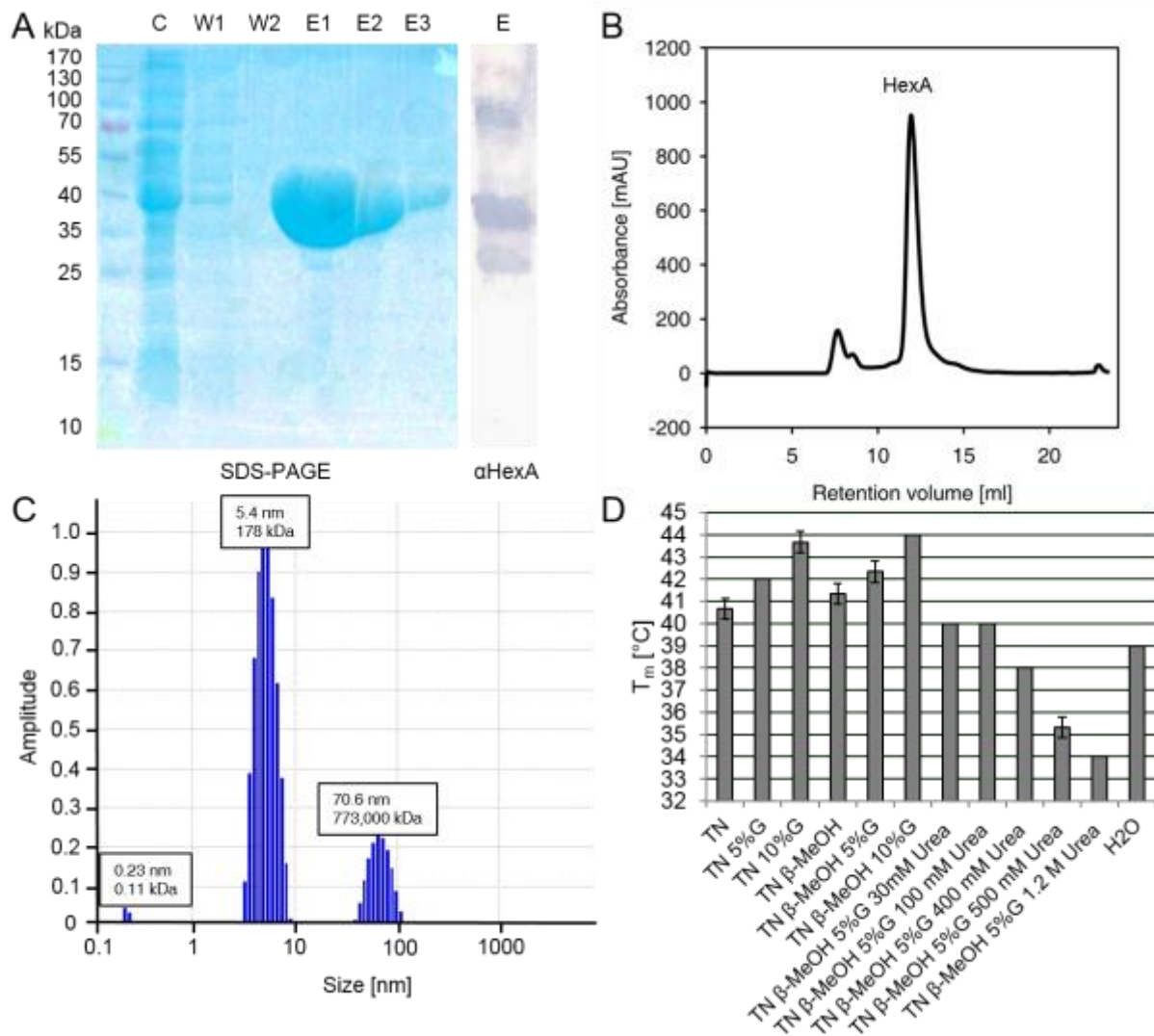


Figure F. Purification and biochemical investigation of HexA-6His. Purification of HexA via Ni-NTA affinity chromatography. Left panel shows a Coomassie blue stained SDS gel; right panel shows a Western blot with αHexA antiserum. C=cytosolic fraction; W1=washing fraction 1; W2=washing fraction 2; E1=elution 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. T_m =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
<i>P. luminescens</i> subsp. laumondi TT01-1°	Wild-type 1° variant, Rif ^R	[1]
<i>P. luminescens</i> subsp. laumondi TT01-2°	Wild type 2° variant, Rif ^R	Lab collection, Dr. David Clarke, University College Cork
<i>P. luminescens</i> TT01-1° Δ hexA	Wild-type 1° variant containing a deletion of <i>hexA</i> (<i>plu3090</i>)	Lab collection, Dr. David Clarke, University College Cork
<i>P. luminescens</i> TT01-1° <i>P</i> _{hexA} - <i>mCherry</i>	TT01-1° harboring <i>P</i> _{hexA} - <i>mCherry</i> reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	[2]
<i>P. luminescens</i> TT01-2° <i>P</i> _{hexA} - <i>mCherry</i>	TT01-2° harboring <i>P</i> _{hexA} - <i>mCherry</i> reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-1° Δ hexA <i>P</i> _{hexA} - <i>mCherry</i>	TT01-1° Δ hexA harboring <i>P</i> _{hexA} - <i>mCherry</i> reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-1° <i>P</i> _{hexA} - <i>hexA-mCherry</i>	TT01-1° harboring <i>P</i> _{hexA} - <i>hexA-mCherry</i> reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-2° <i>P</i> _{hexA} - <i>hexA-mCherry</i>	TT01-2° harboring <i>P</i> _{hexA} - <i>hexA-mCherry</i> reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-1° Δ hexA <i>P</i> _{hexA} - <i>hexA-mCherry</i>	TT01-1° Δ hexA harboring <i>P</i> _{hexA} - <i>hexA-mCherry</i> reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-1° <i>P</i> _{luxC} - <i>mCherry</i>	TT01-1° harboring <i>P</i> _{luxC} - <i>mCherry</i> reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	[2]
<i>P. luminescens</i> TT01-2° <i>P</i> _{luxC} - <i>mCherry</i>	TT01-2° harboring <i>P</i> _{luxC} - <i>mCherry</i> reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study

<i>P. luminescens</i> TT01-1° $\Delta hexA$ P_{luxC} -mCherry	TT01-1° $\Delta hexA$ harboring P_{luxC} -mCherry reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-1° P_{hfq} -mCherry	TT01-1° harboring P_{hfq} -mCherry reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-2° P_{hfq} -mCherry	TT01-2° harboring P_{hfq} -mCherry reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-1° $\Delta hexA$ P_{hfq} -mCherry	TT01-1° $\Delta hexA$ harboring P_{hfq} -mCherry reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-1° P_{pcfA} -mCherry	TT01-1° harboring P_{pcfA} -mCherry reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-2° P_{pcfA} -mCherry	TT01-2° harboring P_{pcfA} -mCherry reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>P. luminescens</i> TT01-1° $\Delta hexA$ P_{pcfA} -mCherry	TT01-1° $\Delta hexA$ harboring P_{pcfA} -mCherry reporter integrated at the <i>rpmE/glmS</i> site, Kan ^R , Gent ^R	This study
<i>E. coli</i> Dh5 α λ pir	<i>recA1</i> , <i>gyrA</i> (<i>lacIZYA-argF</i>) (80d <i>lac</i> [<i>lacZ</i>] M15) <i>pir</i> RK6	[3]
<i>E. coli</i> S17-1 λ pir	Tp ^R Sm ^R <i>recA</i> , <i>thi</i> , <i>pro</i> , <i>hsdR</i> -M+RP4: 2-Tc:Mu: Km Tn7 λ pir	Biomedal S.L. Sevilla, Spain
<i>E. coli</i> ST18	<i>E. coli</i> S17 λ pir $\Delta hemA$	[4]
<i>E. coli</i> BL21 (DE3) Star	F ⁻ <i>ompT</i> <i>hsdS_B</i> (<i>r_B⁻</i> <i>m_B⁻</i>) <i>gal dcm</i> <i>rne131</i> (DE3)	Invitrogen
<i>E. coli</i> JW2284	Kan ^R , BW25113 <i>lrhA::npt</i>	[5]
<i>E. coli</i> $\Delta lrhA$	Removal of the <i>npt</i> cassette in <i>E. coli</i> JW2284 by P1 transduction	Dr. Sophie Brameyer, unpublished
<i>Sh. oneidensis</i> MR1 S79	Wild type isolate	[6]

Table B. Plasmids.

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

	(<i>plu3090</i>) under control of an arabinose inducible promoter	
pBBR1- P_{pcfA} - <i>lux</i>	Gm ^R , <i>luxCDABE</i> under the control of the <i>pcfA</i> (<i>plu4568</i>) promoter	[7]
pBBR1- P_{pcfA} -S1- <i>lux</i>	Gm ^R , <i>luxCDABE</i> under the control of the truncated promoter construct P_{pcfA} -S1	Dr. Sophie Brameyer, unpublished
pBBR- P_{pcfA} -S2- <i>lux</i>	Gm ^R , <i>luxCDABE</i> under the control of the truncated promoter construct P_{pcfA} -S2	Dr. Sophie Brameyer, unpublished
pBBR- P_{lac} - <i>lux</i>	Gm ^R , <i>luxCDABE</i> under the control of the <i>lac</i> promoter	This study
pACYC-Duet1	Cm ^R , Expression vector, IPTG inducible	Novagen®
pACYC- <i>hexA</i>	Cm ^R , <i>hexA</i> (<i>plu3090</i>) in pACYC-Duet1	This study
pACYC- P_{lac} - <i>hexA</i> _ P_{ara} - <i>pluR</i>	Cm ^R , <i>pluR</i> (<i>plu4562</i>) under the control of an arabinose inducible promoter, <i>hexA</i> (<i>plu3090</i>) under the control of an IPTG inducible promoter	This study
pEYFP	Ap ^R , <i>lac</i> -promoter upstream of <i>eYFP</i>	Takara-Clontech, Saint-Germain-en-Laye, France)
pD132	Cm ^R , ori R6K, oriT RK2, <i>sacB</i>	[9]
pDS- <i>hexA</i>	Flanking regions of <i>hexA</i> (<i>plu3090</i>) 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	CTGGTTTCATAATTTGCGC
check-mcherry-ins_rev	GGCCTTCCTTCTCCTTCAC
check-rpmE_fwd	CTCCCAAATAAAGTTTAGG
check-glmS_rev	GTACGTGAATCTGATTTTG
oriT_fwd	CAGGGTTATGCAGCGGAAA
gmRpNPTS_fwd	GATAAGCTGTCAAACATGAGAGTAGCGTATGCGCTCAC
Plac(h)_fwd	ATTGCATTTATCATGGTATATCTCCTTATTAAA
PlacI-Sall_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-Sall_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	TAGCCCTCGAGCTGTGATGATGATGATGATGATGATGATGATG 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	Similarities 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	Arginosuccinate synthase	STAT	+2.0
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(PcfB)

Plu4566	Glycine amidino transferase	STAT	+2.1
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(PcfC)

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