

Title	HexA is a versatile regulator involved in the control of phenotypic heterogeneity of Photorhabdus luminescens
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Publication date	2017-04-27
Original Citation	Langer, A., Moldovan, A., Harmath, C., Joyce, S. A., Clarke, D. J. and Heermann, R. (2017) 'HexA is a versatile regulator involved in the control of phenotypic heterogeneity of Photorhabdus luminescens', PLoS ONE 12(4), e0176535 (23pp). doi: 10.1371/ journal.pone.0176535
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1371/journal.pone.0176535
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Download date	2025-08-02 06:57:52
Item downloaded from	https://hdl.handle.net/10468/3945



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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 (Δ), with reduced production (∇) or overproduced (\Diamond) in the Δ *hexA* mutant and proteins that were completely absent in the Δ *hexA* mutant (\Box) or in the wildtype (\circ) 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 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* $\Delta IrhA$. In *E. coli* $\Delta IrhA$ 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-Para-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 α HexA 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.	Wild-type 1° variant, Rif ^R	[1]
laumondi TT01-1°		
<i>P. luminescens</i> subsp. laumondi TT01-2°	Wild type 2° variant, Rif ^R	Lab collection, Dr. David Clarke, University College Cork
P. luminescens TT01-1°	Wild-type 1° variant containing a	Lab collection, Dr.
ΔhexA	deletion of hexA (plu3090)	David Clarke, University College Cork
P. luminescens TT01-1°	TT01-1° harboring P _{hexA} -mCherry	[2]
P _{hexA} -mCherry	reporter integrated at the <i>rpmE/gImS</i> site, Kan ^R , Gent ^R	
P. luminescens TT01-2°	TT01-2° harboring P _{hexA} -mCherry	This study
P _{hexA} -mCherry	reporter integrated at the $rpmE/glmS$	
D luminococo TT01 1º	TT01 1° A box A barbaring D	This study
P. $ummescens + 101-1$	mChorry reporter integrated at the	This study
Diexa Phexa-incherry	<i>rpmE/gImS</i> site, Kan ^R , Gent ^R	
P. luminescens TT01-1°	TT01-1° harboring P _{hexA} -hexA-	This study
P _{hexA} -hexA-mCherry	mCherry reporter integrated at the	
P luminescens TT01-2°	$TT01-2^\circ$ harboring P_{1} \rightarrow here A_2	This study
P_{1} $hev \Lambda_{-}mCherny$	mCherry reporter integrated at the	This study
r hexA-nexA-moneny	<i>rpmE/gImS</i> site, Kan ^R , Gent ^R	
P. luminescens TT01-1°	TT01-1° $\Delta hexA$ harboring P _{hexA} -hexA-	This study
Δ hexA P _{hexA} -hexA-mCherry	mCherry reporter integrated at the	
	<i>rpmE/gImS</i> site, Kan ^R , Gent ^R	
P. luminescens TT01-1°	TT01-1° harboring P _{luxC} -mCherry	[2]
P _{luxC} -mCherry	reporter integrated at the <i>rpmE/glmS</i>	
	site, Kan ^R , Gent ^R	
P. luminescens TT01-2°	TT01-2° harboring P _{luxC} -mCherry	This study
P _{luxC} -mCherry	reporter integrated at the <i>rpmE/gImS</i>	
	site, Kan ^R , Gent ^R	

P. luminescens TT01-1°	TT01-1° Δ <i>hexA</i> harboring P _{luxC} -	This study
$\Delta hexA P_{luxC}$ -mCherry	mCherry reporter integrated at the	
	<i>rpmE/gImS</i> site, Kan ^R , Gent ^R	
P. luminescens TT01-1°	TT01-1° harboring P _{hfq} -mCherry	This study
P _{hfq} -mCherry	reporter integrated at the rpmE/glmS	
	site, Kan ^R , Gent ^R	
P. luminescens TT01-2°	TT01-2° harboring P _{hfq} -mCherry	This study
P _{hfq} -mCherry	reporter integrated at the rpmE/glmS	
	site, Kan ^R , Gent ^R	
P. luminescens TT01-1°	TT01-1° $\Delta hexA$ harboring P _{hfq} -	This study
ΔhexA P _{hfq} -mCherry	mCherry reporter integrated at the	
	<i>rpmE/gImS</i> site, Kan ^R , Gent ^R	
P. luminescens TT01-1°	TT01-1° harboring P _{pcfA} -mCherry	This study
P _{pcfA} -mCherry	reporter integrated at the rpmE/gImS	
	site, Kan ^R , Gent ^R	
P. luminescens TT01-2°	TT01-2° harboring P _{pcfA} -mCherry	This study
P _{pcfA} -mCherry	reporter integrated at the rpmE/gImS	
	site, Kan ^R , Gent ^R	
P. luminescens TT01-1°	TT01-1° ΔhexA harboring P _{pcfA} -	This study
$\Delta hexA P_{pcfA}$ -mCherry	mCherry reporter integrated at the	
	<i>rpmE/gImS</i> site, Kan ^R , Gent ^R	
<i>E. coli</i> Dh5α λ <i>pir</i>	recA1, gyrA (laclZYA-argF) (80d lac	[3]
	[lacZ] M15) pir RK6	
<i>E. coli</i> S17-1 λpir	Tp ^R Sm ^R recA, thi, pro, hsdR-M+RP4:	Biomedal S.L.
	2-Tc:Mu: Km Tn7 λpir	Sevilla, Spain
E. coli ST18	E. coli S17 λpir ∆hemA	[4]
<i>E. coli</i> BL21 (DE3) Star	F^- ompT hsd $S_B(r_B^- m_B^-)$ gal dcm	Invitrogen
	<i>rne</i> 131 (DE3)	
E. coli JW2284	Kan ^R , BW25113 <i>IrhA::npt</i>	[5]
E. coli ΔlrhA	Removal of the npt cassette in E. coli	Dr. Sophie
	JW2284 by P1 transduction	Brameyer,
		unpublished
Sh. oneidensis 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 _{hexA} -mCherry	Km ^R , Gm ^R , <i>hexA</i> (<i>plu3090</i>) promoter	[2]
	upstream of <i>mCherry</i>	
pPINT-P _{hexA} -hexA-mCherry	Km ^R , Gm ^R , <i>hexA</i> promoter upstream	This study
	of hexA (plu3090)-mCherry	
pPINT-P _{luxC} -mCherry	Km ^R , Gm ^R , <i>luxC (plu2079)</i> promoter	[2]
	upstream of mCherry	
pPINT-P _{hfq} -mCherry	Km ^R , Gm ^R , <i>hfq (plu4581)</i> promoter	This study
	upstream of mCherry	
pPINT-P _{pcfA} -mCherry	Km ^R , Gm ^R , <i>pcfA (plu4568)</i> promoter	This study
	upstream of mCherry	
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-	[8]
	terminal HisTag	
pBAD24- <i>hexA</i>	Ap ^R , <i>hexA-</i> 6His (<i>plu3090</i>) in pBAD24	This study
	with a C-terminal HisTag	
pCOLA-ppyS-His-pluR	Km ^R , <i>ppyS (plu4844</i>) and 6His- <i>pluR</i>	Dr. Sophie Brameyer,
	(<i>plu4562</i>) in pCOLA, IPTG inducible	unpublished
pBAD24-P _{ara} - <i>pluR_</i> P _{lac} -	Ap ^R , <i>pluR</i> (<i>plu4562</i>) under the control	This study
hexA	of an arabinose inducible promoter,	
	hexA (plu3090) under the control of	
	an IPTG inducible promoter	
pBAD24-P _{lac} -pluR_P _{ara} -	Ap ^R , <i>pluR</i> (<i>plu4562</i>) under the control	This study
hexA	of an IPTG inducible promoter, hexA	

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

Table C. Oligonucleotides.

Primer name	Sequence (5`-3`)
PhexA-BamHI_fwd	GCTGGATCCTCTTACCTTATCTTGGTAAA
hexA-Xmal _rev	GCTCCCGGGCTCATCAATAATATCGTCATCATCA
Phfq-Nhel_fwd	GCGGCTAGCTCACTGAACTGACTACATTG
Phfq-BamHI_rev	GCTGGATCCTCTATATTTTCCTTATTTTGTT
PpcfA-Nhel_fwd	AATGGAGCTAGCAGCAGAATTCGGGTTAGTTATCTATGC
PpcfA-Xmal_rev	ACTAAGCCCGGGACCAGCTTTATCCCTTATGTC
check-mcherry_ins_fwd	CTGGTTTCATAATTTCGCC
check-mcherry-ins_rev	GGULTIUTUTUTUAU
check-rpmE_fwd	CTCCCAAATAAAGTTTAGG
check-glmS_rev	GTACGTGAATCTGATTTTG
oriT_fwd	CAGGGTTATGCAGCGGAAA
gmRpNPTS_fwd	GATAAGCTGTCAAACATGAGAGTAGCGTATGCGCTCAC
Plac(h)_fwd	ATTGCATTTATCATGGTATATCTCCTTATTAAA
Placl-Sall_rev	GCTGTCGACTCACTGCCCGCTTTCCAGTC
hexA_fwd	ATGATAAATGCAAATCGTC
hexA-PstI-rev	GCTCTGCAGTTACTCATCAATAATATCG
pBAD24_seq_fwd	GCCGTCACTGCGTCTTTTACTGG
pBAD24_seq_rev	CGCTACGGCGTTTCACTTCTG
hexA-EcoRI_fwd	GCTGAATTCATGATAAATGCAAATCGTCC
hexA-Ndel_rev	GCGCATATGCTCATCAATAATATCGTCATCATC
Plac-PluR_fwd	TCTTCAAAGCTTGCGGCCGCATAATG
PluR-Pstl_rev	GCGCTGCAGGTTATATGATTAGATTATATGCTATTGC
lacl_fwd	CAAGCTTTGAAGATCGAATGGCGCAAAACCTT
lacl-Sall_rev	GCTGTCGACTCACTGCCCGCTTTCCAGTC

check-PlachexA_fwd	CTACCAGAGAAGTTGAAGT
hexA-Ncol_fwd	GCTCCATGGATGATAAATGCAAATCGTCC
hexA-Sall_rev	GCGGTCGACTTACTCATCAATAATATC
check-pACYC_fwd	ATTCACCACCCTGAATTGA
check-pACYC_rev	CTAGTTATTGCTCAGCGGT
araCPluR_fwd	GCGCATATGACTCCGTCAAGCCGTCAA
pluR-Xhol_rev	TAGCCCTCGAGCTGTGATGATGATGATGATGATGATGATGATG
	ACGACCTTCGATATGGCCGCTTATATGATTAGATTATATGC
PpcfA-Btn_fwd	ΤΑΤΤΤGTCTTTATAATGATAAT
PpcfA_rev	ACCAGCTTTATCCCTTATGTC
sacB-Btn_fwd	GCAGAAGTTTTTGACTTTCTTG
sacB_rev	ACATCTGACGGAAAAATCCGT
Plac-Nhel_fwd	GCGGCTAGCGCGCAACGCAATTAATGTG
Plac-BamHI_rev	CGCGGATCCAGCTGTTTCCTGTGTGAAA
check-pBBR-Plac_fwd	CCGTCGTATTAAAGAGGGG
FA_hexA_fwd	GAATTGTTGTTGTTTTTA
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	ΔhexA/wild-type
Plu0184	Role in Carbapenem biosynthesis		+4.2
(CpmC)			
Plu0261	Similiarities with type 1 fimbrial	EX	n.d. in ∆ <i>hexA</i>
	protein precursor		
Plu0269	Unknown, hypothetical secreted	EX	n.d. in ∆ <i>hexA</i>
	protein		
Plu0885	Pyocin S3 protein, "killer protein"	EX	n.d. in ∆ <i>hexA</i>
Plu0888	Colicin/Pyocin protein, "killer protein"	EX	n.d. in ∆ <i>hexA</i>
Plu1395	Cystein Synthase A	STAT	-1.7
Plu1561	Ca2+-dependent cell adhesion	STAT	-2.6
	molecule		
Plu1840	unknown	STAT	-1.6
Plu2016	PAS4-LuxR regulator	EX	n.d. in ∆ <i>hexA</i>
Plu2248	Carbonic anhydrase	EX	+4.0
Plu3102	methyltransferase	EX	n.d. in WT
Plu3104	unknown	EX	n.d. in WT
Plu3110	Succinylornithine transaminase	STAT	-1.4
(ArgM)			
Plu3254	Hcp family T6SS protein CtsH1	EX	-2.7
Plu3622	dihydrolipoamide acetyltransferase;	STAT	-2.2
(AceF)	pyruvate dehydrogenase subunit E2		
Plu3739	Aldehyde Dehydrogenase B	EXP	+1.8
(AldB)			
Plu3795	unknown	STAT	+2.4
Plu4078	Dimethylmenaquinone	EXP	n.d. in ∆ <i>hexA</i>
	methyltransferase		
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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