Supplemental figures



Fig. S1. Cultivation of *C. glutamicum* strains on minimal medium (MM) with 5 mM PAA (A) or 5 mM glucose as carbon source (B). MM agar plates with glucose were incubated for 24 h at 30 °C, while plates with PAA were incubated for 48 h at the same temperature. The growth of AS 1.542 and ATCC 13032/pEC-K18*mob2::paa* was also observed in MM broth, and same results were observed. Labels: 1, *C. glutamicum* AS 1.542; 2, *C. glutamicum* ATCC 13032/pEC-K18*mob2::paa*; 3, *C. glutamicum* ATCC 13032/pEC-K18*mob2*; 4, *C. glutamicum* ATCC 13032.



Fig. S2. Identification of probable transcription start sites (TSS) and promoter regions in the *paa* gene cluster of the strain AS 1.542. Letters A to F refer to region that spans the probable TSS and promoter of *paaK* (A), *paaA* (B), *paaR* (C), *paaI* (D), *paaY* (E), and *paaZ* (F) genes, and their sequence is shown in the red box. The TSS is indicated by gray background and the -10 region by blue background. The sequences of TSS and -10 regions are indicated outside the red box. Values for perfect and best matching reads (see Table 2, manuscript) are indicated by green and yellow color, respectively.



0 0 10 25 50 100 250 500 µM Effector

Fig. S3. Effect of Acetyl-CoA, PAA and PA-CoA on PaaR binding to the Cy3-labeled DNA probe for *paaA*. Whether 5 pmol PaaR protein was added to the reaction mixture is indicated by – and + above the gel picture. Acetyl-CoA, PAA and PA-CoA were added to the reaction mixture in the indicated concentration gradient.

Supplemental Table S1. Bacteria strains, plasmids and primers used in this study

Strain, plasmid or primer	Relevant features or sequences ^a	Source, reference or note
C. glutamicum strains		
Strain AS 1.542	Wild type strain AS 1.542	(1)
AS $1.542/paaR^{\Delta HTH}$	Strain AS 1.542 with deletion of the HTH domain of <i>paaR</i> gene	this study
ATCC 13032	Type strain	(2)
ATCC 13032/		
pEC-K18mob2::paa	ATCC 13032 carrying pEC-K18mob2::paa	this study
E. coli strains		NED
EK2300 ED2566/mTVD1.umg gP	NEB IMPACT KIL	NEB this study
EK2300/p1AB1::paak	Paak protein expression strain carrying p1Xb1paak	uns study
Plasmids		
pK18mobsacB	cloning vector for allelic exchange. Km ^R	(5)
pK18mobsacBpaa $R^{\Delta HTH}$	nK18 <i>mobsacB</i> carrying <i>pagR</i> gene with deletion of its HTH domain Km ^R	this study
pTXB1	NEB IMPACT Kit. Amp ^R	NEB
pTXB1::paaR	pTXB1 derivative for expression of PaaR protein. Amp^{R}	this study
pEC-K18mob2	C. glutamicum cloning vector, Km^{R}	(3)
pEC-K18mob2::paa	pEC-K18mob2 carrying the whole cluster of paa genes, Km ^R	this study
pETCRP	pET-28a derivative containing the $glxR$ gene from C. $glutamicum$, Km ^R	(4)
Primers ^b		
725_DL_HTH_A	A <u>GGTCTC</u> GAATTCTCCGGTCAGTAAGTTGTGAG (Eco31I)	To generate
725_DL_HTH_B	GGATCCATTGCCGTTCTGTG	pK18mobsacB::paa
725_DL_HTH_C	CACAGAACGGCAATGGATCCCTTGGGAAATGCTCGTTGGA	$R^{\Delta HTH}$
725_DL_HTH_D	A <u>GGTCTC</u> GGATCCGCTCCCCTGGTGGAATTACA (Eco31I)	
paa_gal		
paa_ga2		т (
paa_vector_gal		To generate
paa_vector_gaz	GAIGUGAIIUGIAIGGGGIIUUIGUUAIIIGUUAUGIII	pec- k 18mob2.:paa
723 RT F	CGTAGCCAATGACGAAGGTT	
723_RT_R	CAGCACTCCAAGCTGGTTAT	
724 RT F	GTCAGTGGGGTGATGACAAG	
724 RT R	CTCCGAACCATGGAGTGAAG	
725 RT F	TAATTCTGCGGTGGTGTCTG	
725_RT_R	TTCGACTTCGTGGCAACTCT	
727_RT_F	TCGTTGAAGGCCATGGGTAT	
727_RT_R	CGGCGCAATGAAATGGATCT	
728_RT_F	GCCTGAGGCTAACTGGATTT	
728_RT_R	GTGCGAGCTGGGTAATTGAA	
729_RT_F	CTCAAACCGCGGATTATCCC	
729_RT_R	GTAGGTTGCATGGCGGTAAG	
730_R1_F		
/30_RI_R 721_PT_E		
/31_R1_F 721 PT P		
731_R1_R 732 PT F	GCCAGTACGTTGCTCTACGA	
732_RT_R 732_RT_R	GAGGCTGGTGACGTGAATCT	
732_RT_R 733 RT F	GCTCGGGACGAAACATCAAA	
733 RT R	ATCAAGGGTTGCGCCTAAGT	
734 RT F	TTCGCCCTGATGATCTAGCA	
734_RT_R	GATTCCACCATCGCAGTAGC	
735_RT_F	TGCCTCTGGTGCAGATATCG	
735_RT_R	GCTTCTGGCTGACCAAACTT	
736_RT_F	GCGGTTCCTGAGTCTTTTGA	
736_RT_R	TGGGTAGCCACCACAATTTC	
737_RT_F	GCCTGCGTCATCATGAAGAA	
737_RT_R	GATGGCAACCGACAGTCTTT	
738_RT_F	CTCCCCAACACCAATGTGAT	_
738_RT_R	TGCTTCTGCGGTGATGTAAC	For RT-qPCR
/40_KI_F		analysis of <i>paa</i>
/4U_K1_K	ILLUIULAALILLUIAIAL	cluster genes

725E_int1

GGTGGT<u>CATATG</u>ATGACTCCAACGAGCATTTC (NdeI)

To generate

725E_int2	GGTGGTT <u>GCTCTTC</u> CGCAAGTGCTGGGTGTGGCTGTTT (SapI)	pTXB1::paaR
724_upstream_F ^b	GGTCCATTGTGGGTGGAAGA	
724_upstream_R	CCACACCCAGCACTTAGACT	
725_upstream_F	CCGGTGGCGGGAAGCGCTTG	
725_upstream_R ^b	CCAGGTCAGACTCAATTTGT	
728_upstream_F	TTCGGGGAATGTCTCGAACT	
728_upstream_R ^b	GCTGTATCTGCGTTTGTGTC	To generate Cy3
738_upstream_F	CAGTTCGCCGAGCTGAATAG	labeled upstream
738_upstream_R ^b	CCCACTGTCCCTGAAGGTAG	DNA probes of
hemH_EMSA_1	CGTGGTACGCCTCATTTAGT	paaK, paaR/I, paaA,
hemH_EMSA_2 ^b	CATCCGATGTGCGTTCATTC	paaY/Z and $hemH$
724 oligo F	TTTCCATTAACTTACCGACCTTCCGGTCAGTAAGTTGTGA	
724 oligo R	TCACAACTTACTGACCGGAAGGTCGGTAAGTTAATGGAAA	
725_oligo_F	TTTCTCAACCATAACCGGGAAAGGGCCTGGTTATGTAGAA	
725_oligo_R	TTCTACATAACCAGGCCCTTTCCCGGTTATGGTTGAGAAA	
728_oligo_F	TCTGGATATCCTGACCGAACGATCGGTAATTAAATGATTC	To generate
728_oligo_R	GAATCATTTAATTACCGATCGTTCGGTCAGGATATCCAGA	competition DNA
738_oligo_F	ACCCATATTACTTACCGTACGGTCGGTAAGTGTAATTATT	fragments of paaK,
738_oligo_R	AATAATTACACTTACCGACCGTACGGTAAGTAATATGGGT	paaR/I, paaA,
argC_EMSA1	CAAAAATTTATGCATGAATAATTTGCATGATCATGCATAA	paaY/Z and $argC$
argC_EMSA2	TTATGCATGATCATGCAAATTATTCATGCATAAATTTTTG	for PaaR EMSA
724_oligo_GlxR_F ^b	TTTCAAAGCACGTGTAGCGTAGCTCACAACTTACTGACCG	To generate Cy3
724_oligo_GlxR_R	CGGTCAGTAAGTTGTGAGCTACGCTACACGTGCTTTGAAA	labeled DNA
728_oligo_GlxR_F ^b	GATCGCGGAAAGATTGACCCAGATCACACTATCTGGATAT	fragments of paaK,
728_oligo_GlxR_R	ATATCCAGATAGTGTGATCTGGGTCAATCTTTCCGCGATC	paaA and $paaY/Z$
738_oligo_GlxR_F ^b	CTTCACCAAAAGTGTGAAGTGCGTTATACTGACCCATATT	for GlxR EMSA
738_oligo_GlxR_R	AATATGGGTCAGTATAACGCACTTCACACTTTTGGTGAAG	

^a Restriction enzyme cut sites are underlined.

^b These primers are labeled with Cy3 fluorescence.

References for supplemental Table S1

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