

Increased acetate production in *Synechocystis* sp. PCC 6803 strain engineered with an operon of phosphoketolase and phosphotransacetylase and further overexpression of acetate kinase

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Additional File 1

Tables S.1-S.4

References

Table S.1: Genetic engineered *Synechocystis* sp. PCC 6803 strains used in this study

<i>Synechocystis</i> strain	Relevant Genotype	References
WT_PKPa_Δacs	Δacs:: (PtrcRiboJ- PKPa -T)Km ^R	Roussou <i>et al.</i> , 2025
WT_PKPa_Δacs_Δddh	Δacs:: (PtrcRiboJ- PKPa -T)Km ^R , Δddh::Sm ^R	This study
WT_PKPa_Δacs_Δddh_Ptrc_BsPta	Δacs:: (PtrcRiboJ- PKPa -T)Km ^R , Δddh:: (PtrcRiboJ- BsPta -T)Sm ^R	This study
WT_PKPa_Δacs_Δddh_PsbA2_BsPta	Δacs:: (PtrcRiboJ- PKPa -T)Km ^R , Δddh:: (PsbA2- BsPta -T)Sm ^R	This study
WT_PKPa_RBS_Bspta_Δacs	Δacs:: (PtrcRiboJ- PKPa -RBS- BsPta -T)Km ^R	This study
WT_PKPa_SL_Bspta_Δacs	Δacs:: (PtrcRiboJ- PKPa -SL*- BsPta -T)Km ^R	This study
WT_PKPa_ML_Bspta_Δacs	Δacs:: (PtrcRiboJ- PKPa -ML* ¹ - BsPta -T)Km ^R	This study
WT_PKPa_LL_Bspta_Δacs	Δacs:: (PtrcRiboJ- PKPa -LL* ² - BsPta -T)Km ^R	This study
WT_PKPa_RL_Bspta_Δacs	Δacs:: (PtrcRiboJ- PKPa -RL* ³ - BsPta -T)Km ^R	This study

*Part: BBa_K243004 Short Linker (Gly-Gly-Ser-Gly)

*¹Part: BBa_K243005 Middle Linker (Gly-Gly-Ser-Gly) x2

*²Part: BBa_K243006 Long Linker (Gly-Gly-Ser-Gly) x3

*³Rigid linker (Leu-Gln-Ser-Arg-Leu-Glu), (Oliveira and Lindblad, 2011)

Antibiotic markers: Km = kanamycin, Sm = spectinomycin

The strong Terminator BBa_B0015 is indicated by T in the expression unit

With bold are indicated the expressed genes

Table S.2: Plasmids targeting *Synechocystis* sp. PCC 6803 genome used in this study

Backbone Vector	Integration site	Antibiotic resistance/genetic construct	Reference
IVE Δacs-PKPa-RBS-BsPta	<i>acs</i> (<i>sll0542</i>)	Km/ PtrcRiboJ- PKPa -RBS- BsPta -T	This study
IVE Δacs-PKPa-SL-BsPta	<i>acs</i> (<i>sll0542</i>)	Km/ PtrcRiboJ- PKPa -SL*- BsPta -T	This study
IVE Δacs-PKPa-ML-BsPta	<i>acs</i> (<i>sll0542</i>)	Km/ PtrcRiboJ- PKPa -ML* ¹ - BsPta -T	This study
IVE Δacs-PKPa-LL-BsPta	<i>acs</i> (<i>sll0542</i>)	Km/ PtrcRiboJ- PKPa -LL* ² - BsPta -T	This study
IVE Δddh	<i>ddh</i> (<i>slr1556</i>)	Sm	This study
IVE Δddh-Ptrc_BsPta	<i>ddh</i> (<i>slr1556</i>)	Sm/ PtrcRiboJ- BsPta -T	This study
IVE Δddh-PsbA2_BsPta	<i>ddh</i> (<i>slr1556</i>)	Sm/ PsbA2- BsPta -T)	This study

*Part: BBa_K243004 Short Linker (Gly-Gly-Ser-Gly)

*¹Part: BBa_K243005 Middle Linker (Gly-Gly-Ser-Gly) x2

*²Part: BBa_K243006 Long Linker (Gly-Gly-Ser-Gly) x3

*³Rigid linker (Leu-Gln-Ser-Arg-Leu-Glu), (Oliveira and Lindblad, 2011)

Antibiotic markers: Km = kanamycin, Sm = spectinomycin

The strong Terminator BBa_B0015 is indicated by T in the expression unit

With bold are indicated the expressed genes

Table S.3: Plasmids and *Synechocystis* sp. PCC 6803 strains used in this study

Plasmid	Promoters and enzymes	<i>Synechocystis</i> strains	References
PsbA2+ach	<i>PpsbA2</i> , acetyl-CoA hydrolase (<i>ach</i>)	WT_ PKPa _RBS_ BsPta _Δacs +PsbA2_ ach	Roussou <i>et al.</i> , 2025/This study
PsbA2+acka	<i>PpsbA2</i> , acetate kinase (<i>acka</i>)	WT_ PKPa _RBS_ BsPta _Δacs +PsbA2_ acka	Roussou <i>et al.</i> , 2025/This study

With bold are indicated the expressed genes

Table S.4: Primers used in this study

Name	Sequence	Reference
US_acs_Pho_F	/5Pho/TTTCCACTTCACTTGGTT	Roussou <i>et al.</i> , 2025
US_acs_Ptrc_R	GAGCGCTCACAATTGTCAACAGCTCGGTTCTCCGTC AAAGTCT	Roussou <i>et al.</i> , 2025
DS_acs_R	CAAATTAGCCAAACCCAC	Roussou <i>et al.</i> , 2025
DSsll0542_linker_F	GTTAATTGGTTGTAACTGGCAGAGC	This study
US_ddh_F	/5Pho/AAACCACTGGGCCAGTAG	Roussou <i>et al.</i> , 2025
US_ddh_Ptrc_R	GAGCGCTCACAATTGTCAACAGCTCGACGATTATGG GAAGTAGTT	Roussou <i>et al.</i> , 2025
DS_ddh_Sm_F	TGGTGAGAATCCAAGCCTCGAGCTGGGTTAGAAAAT ATCAATGTAACT	Roussou <i>et al.</i> , 2025
DS_ddh_R	CGGACTATTTGGTAGAACA	Roussou <i>et al.</i> , 2025
Ptrc_F	/5Phos/GAGCTGTTGACAATTGTGAGCG	Roussou <i>et al.</i> , 2025
Ptrc_acs_F	CCATTAAAGACTTTGACGGAGAACCGAGCTGTTGAC AATTGTG	Roussou <i>et al.</i> , 2025
Ptrc_RiboJ_R	CATTTTTTTCCTCCTTCTAGT	Roussou <i>et al.</i> , 2025
Ptrc_ddh_F	TTCTAAACTACTTCCCATAATCGTCGAGCTGTTGAC AATTGTG	Roussou <i>et al.</i> , 2025
PsbA2_ddh_F	AACTACTTCCCATAATCGTCTCCGCCAGGTAAACTC TTC	This study
KmR_F	GGTTGCATTTCGATTCTCTGTT	Roussou <i>et al.</i> , 2025
Km_Pho_R	/5Pho/CAATTCTGATTAGAAAACTCATCGAGC	This study
SmR_ddh_R	TAGTTAACATTGATATTTTCTAACCCAGCTCGAGGC TTGGATT	Roussou <i>et al.</i> , 2025
SmR_R	/5Pho/CAGCTCGAGGCTTGGATT	Roussou <i>et al.</i> , 2025
PK_PtrcRiboJ_F	TAGAAGGAGGAAAAAATGATGTGGAGTCATCCTC AG	Roussou <i>et al.</i> , 2025
PK_nsc_Pho_R	/5Pho/ACGCAATAAGGCGGGATCGCC	This study
pkpa_mid_R	CGGTATGGGGATTACATG	Roussou <i>et al.</i> , 2025
pkpa_seq_R	CAGGCGGACACCACGGCTTCT	Roussou <i>et al.</i> , 2025
Pta_colony_R	ATGGCCACGCGGGGTTCA	Roussou <i>et al.</i> , 2025
SL_BsPta_F	GGTGGTAGTGGTGGCGACTTGTTTAGCACTGT	This study
ML_BsPta_F	GGTGGGAGTGGGGGAGGAAGCGGTGCCGACTTGTT TAGCACTGT	This study
LL_BsPta_F	GGTGGTAGTGGTGGTGGGAGTGGGGGAGGA	This study
RL_BsPta_F	CTGCAAAGCCGCCTAGAAGCCGACTTGTTTAGCACT GT	This study
BsPta_EcoRI_RBS_F	ATTTGGAATTCAGTAGATAGTGGAGGTACTAGAATG CATCATCATCATCATGC	This study
BsPta_XhoI_PstI_R	TAACTCGAGCTGCAGTTACAAAGCCTGGGCAGC	This study
BsPta_Pho_R	/5Pho/TTACAAAGCCTGGGCAGC	This study

SmR_colony_F	GGC GAT GAG CGA AAT GTA GT	Roussou <i>et al.</i> , 2025
US_acs_colony_R	GGACAGTGGGGAAAAGATCA	Roussou <i>et al.</i> , 2025
DS_acs_colony_R	TTTCTTCATCCGTGGGACTC	Roussou <i>et al.</i> , 2025
US_ddh_colony_R	CTCACACTGGGGCAACTGTA	Roussou <i>et al.</i> , 2025
DS_ddh_colony_R	CGAACAGATAATCGGCATCA	Roussou <i>et al.</i> , 2025
SmR_integr_R	CAA CGC TAT GTT CTC TTG CTT T	Roussou <i>et al.</i> , 2025
UUS_acs_F	ACATCGGCTGGATGATGTTT	Roussou <i>et al.</i> , 2025
DDS_acs_F	GGGCTTTGTTATTGGCTGAG	Roussou <i>et al.</i> , 2025
UUS_ddh_F	GCGACAGGAAAAGGAACAAA	Roussou <i>et al.</i> , 2025
DDS_ddh_F	GGAGCACAGCCTTGGATAAG	Roussou <i>et al.</i> , 2025
acs_integr_F	AGCAATCCGGAGAAAGTTT	Roussou <i>et al.</i> , 2025
acs_integr_R	AATAAAGGCTCGGATGGC	Roussou <i>et al.</i> , 2025
ddh_integr_F	CTCAACCTTGATACCGCTATTTTAG	Roussou <i>et al.</i> , 2025
ddh_integr_R	ATGGGGACAGATTACTTGGTAAAGTT	Roussou <i>et al.</i> , 2025
Term_backbone_F	TGCAGCCAGGCATCAAATAAAAC	This study

References:

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