

SUPPLEMENTARY

Synthetic consortia towards photosynthetically derived acetate for heterotrophic production of 1-butanol

Stamatina Roussou and Peter Lindblad*

stamatina.roussou@kemi.uu.se

peter.lindblad@kemi.uu.se

*Microbial Chemistry, Department of Chemistry-Ångström Laboratory, Uppsala University,
Box 523, SE-75120*

Uppsala, Sweden

* Corresponding author

Appendix A

Tables S.1-S.2

Figures S.1-S.3

Table S.1 Primers (a) and gBlock (b) used in this study

a. Primers

Name	DNA sequence	Reference
PthI_XbaI_F	GCCGCTTCTAGAGGCAGCCAATAAGAAGGAGAAG	This study
PthI_OEBF_R	CTCCCAATTTTTTTGTCATCGCTCATCTTGTCATCGTCATCCTTGTA	This study
BF_PthI_F	ATGAGCGATGACAAAAAAATT	This study
BF_colony_R	GGCCATTTCAAAGCGCTGAA	This study
ppt_end_F	ATCCGAATTCAGGAGGTAAAAAATGAGCC	This study
ppt_end_R	ATGGCTCATTTTTTTACCTCCTGAATTCGG	This study
SpeI_car_R	CCGCTACTAGTATTACAGCAGGCCCAGCAG	This study
VR_SR_R	AACCGAAGTAATCTTTT	This study
RSF1010_seq	CCCTGCTGGGTTTGCAGG	This study
SRV_seq_R	GCTGGACAGCGAAACCGC	This study
VR_SR_F	AAAATAGGCGTATCACGAG	This study
car_colony_F	TGAATTTGTGGATTGGCTGA	This study

b. gBlock for Pem7

GCAGCCAATAAGAAGGAGAAGGCGATTGTTGACAATTAATCATCGGCATAGTATATCGG
CATAGTATAATACGACAAGGTGAGGAACTAAACCCCTAGGGCCCAAGTTCACCTAAAAA
GGAGATCAACAATGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCTAAGGAGGTTT
TCTAATGGACTACAAGGATGACGATGACA

With *Red* the Pem7 promoter is highlighted

With *Green* the BCD2 region is highlighted

With *Blue* the Flag Tag is highlighted

Table S.2 Plasmids and strains used in this study

Plasmid	Promoters and genes encoding for the enzymes	Strains	References
pPMQAK1	<i>Empty vector</i>	<i>E. coli</i> _pPMQAK1	Huang et al. 2010
pET-TPC4	<i>PT7, aat_{BF}*-ppt_{BS}^{*1}-car_{MM}^{*2}</i>	<i>E. coli</i> _pET_TPC4	Pásztor et al. 2015
Pthl_ButOH	<i>Pthl, aat_{BF}, ppt_{BS}, car_{MM}</i>	<i>E. coli</i> _Pthl_ButOH <i>P. taiwanensis</i> _Pthl_ButOH	This study
Pem7_ButOH	<i>Pem7, aat_{BF}, ppt_{BS}, car_{MM}</i>	<i>E. coli</i> _Pem7_ButOH <i>P. taiwanensis</i> _Pem7_ButOH	This study

**aat_{BF}*: gene encoding for acyl-ACP thioesterase from *Bacteroides fragilis*

*¹*ppt_{BS}*: gene encoding for phosphopantetheinyl transferase maturation factor from *Bacillus subtilis*

*²*car_{MM}*: gene encoding for carboxylic acid reductase from *Mycobacterium marinum*

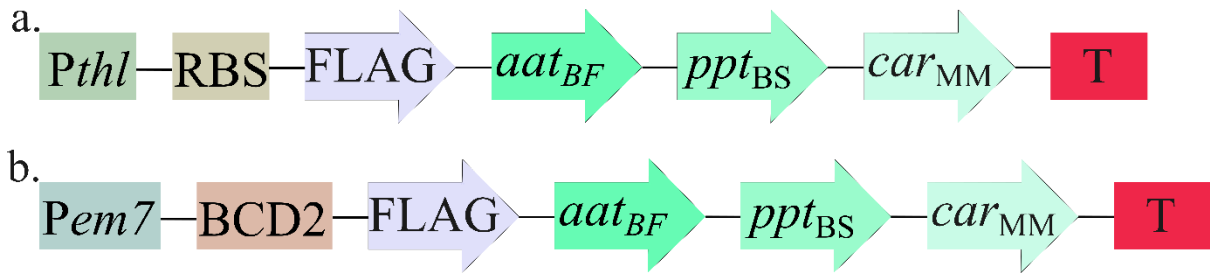


Figure S.1: Schematic representation of the genetic constructs developed in this study. *Pthl* (a) and *Pem7* (b) represents the two different promoters tested in this study. In (a), an RBS is followed, the Flag tag and the genetic construct for the expression of the 1-butanol pathway. The genes included are *aat*_{BF}; acyl-ACP thioesterase from *Bacteroides fragilis*, *ppt*_{BS}; phosphopantetheinyl transferase maturation factor from *Bacillus subtilis* and *car*_{MM}; carboxylic acid reductase from *Mycobacterium marinum*. The T represents his operon terminator located on the pPMQAK1 plasmid. In (b), the BCD2 region is following the *Pem7* promoter instead of the RBS. The rest of the construct remained the same.

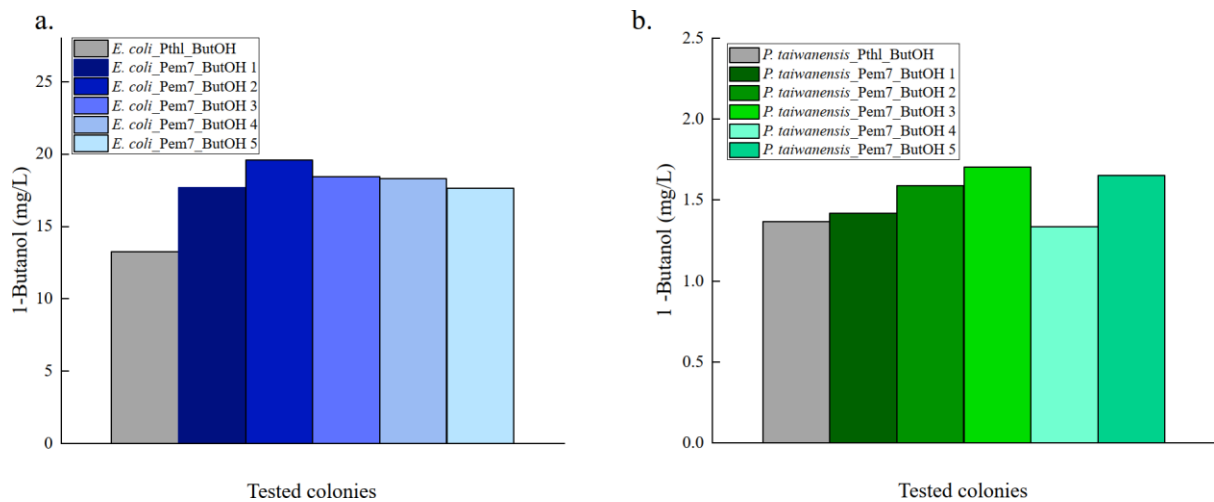
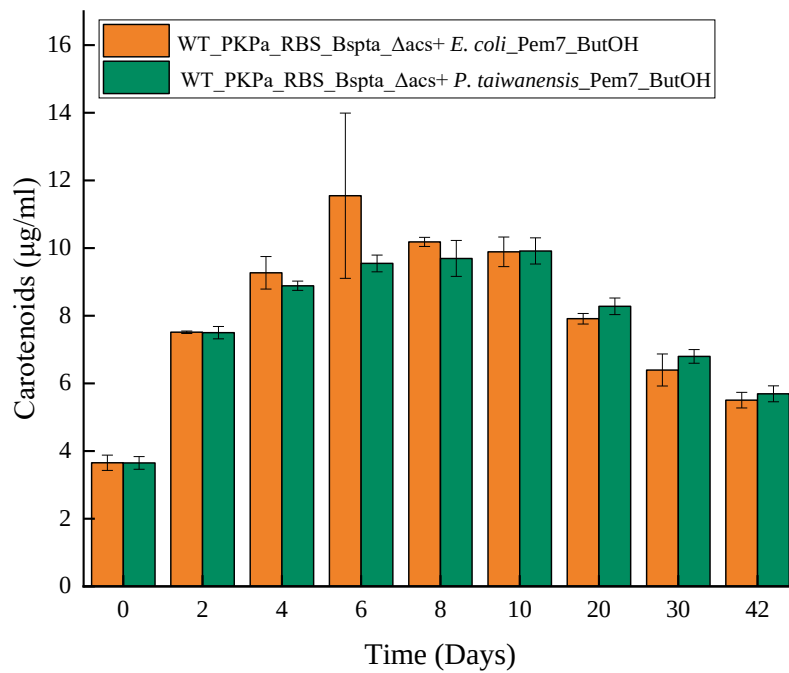


Figure S.2: Screening experiment for individual colonies of *E. coli* BL21 (a) and *P. taiwanensis* VBL120 (b) after transformation with Pem7_ButOH compared to strains containing the plasmid Pthl_ButOH. The colonies were grown in a closed system overnight and then the 1-butanol production was quantified.

a.



b.

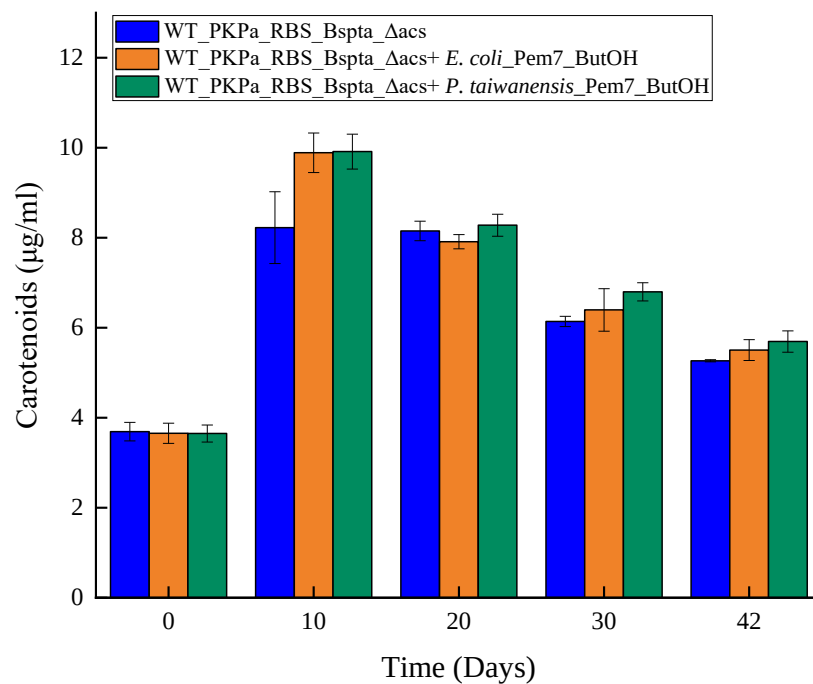


Figure S.3: Carotenoid concentration of *Synechocystis* PCC 6803 WT_PKPa_RBS_BsPta_Δacs (blue) grown in synthetic consortia with either *E. coli*_Pem7_ButOH (orange) or *P. taiwanensis*_Pem7_ButOH (green). The carotenoid concentration is shown through selected dates of the experiment (a) while it was also compared to the monoculture (b). 10% of the volume (2.5 ml) was sampled every 2 days

from day 0 to day 30 while from day 30 to day 42 the culture was sampled every 4 days and replaced with 2.5 ml of CoBG11 containing 500 mM NaHCO₃ (final concentration 50 mM) and kanamycin and the pH was adjusted at 7 with the addition of 37% HCl. The consortia were growing under light intensity 65 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and the temperature was 30°C. The results are represented as mean $\pm$ SD of the three biological replicates.

References

- Huang H-H, Camsund D, Lindblad P, Heidorn T (2010) Design and characterization of molecular tools for a synthetic biology approach towards developing cyanobacterial biotechnology. *Nucleic Acids Res* 38:2577–2593. <https://doi.org/10.1093/nar/gkq164>
- Pásztor A, Kallio P, Malatinszky D, Akhtar MK, Jones PR (2015) A synthetic O₂-tolerant butanol pathway exploiting native fatty acid biosynthesis in *Escherichia coli*. *Biotechnol Bioeng* 112(1):120–128. <https://doi.org/10.1002/bit.25324>