Supplementary Material:

Engineering a cyanobacterial cell factory for the production of lactic acid

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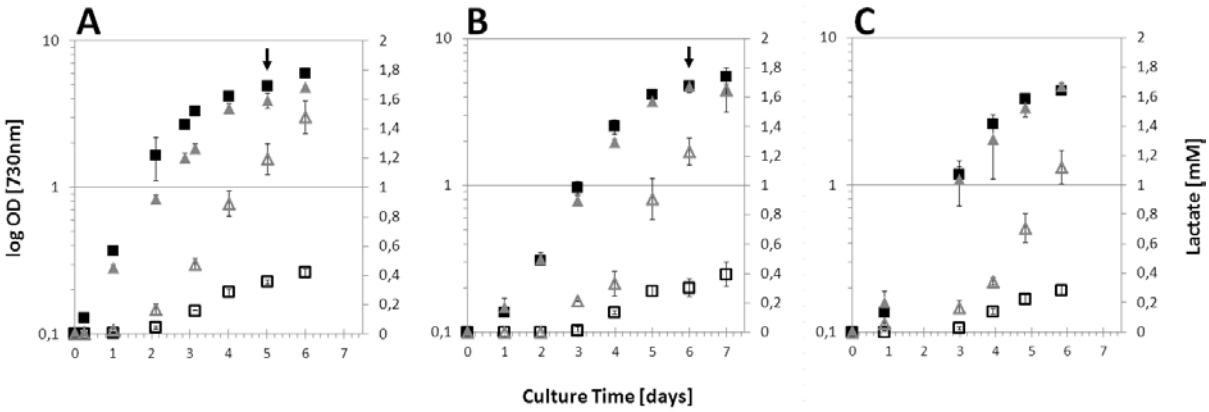
Calculations:

The following values were taken from the literature: The maximal rate of fixation of carbon of 3.70 mmol/gDW/h (2, 3) was derived from the maximal growth rate of the organism of 0.085 h^{-1} (1). This translates into 0.085 g/gDW/h. Assuming an average cellular composition of $C_4H_7O_2N$ (corresponding to a M_W of 101,10) we derive a $Q_{biomass}$ of 0.86 mmol biomass/gDW/h, which in turn, when taking into account that 4 mole of CO_2 are necessary to form 1 mole of $C_4H_7O_2N$, leads to a CO_2 fixation rate (Q_{CO2}) of 3.44 mmolC/gDW/h; Nonetheless as maximal fixation rate we adopt the slightly higher rate reported in (3), which was derived from their own experimental data. The determined capacity of CO_2 fixation for a *Synechocystis* strain at its respective maximum growth rate can be expressed as minimally required enzymatic activity of the CO_2 fixing enzyme Ribulose-1,5-Bisphosphate Carboxylase Oxygenase. Enzymatic activities (Table 2) can be expressed in relation to that activity.

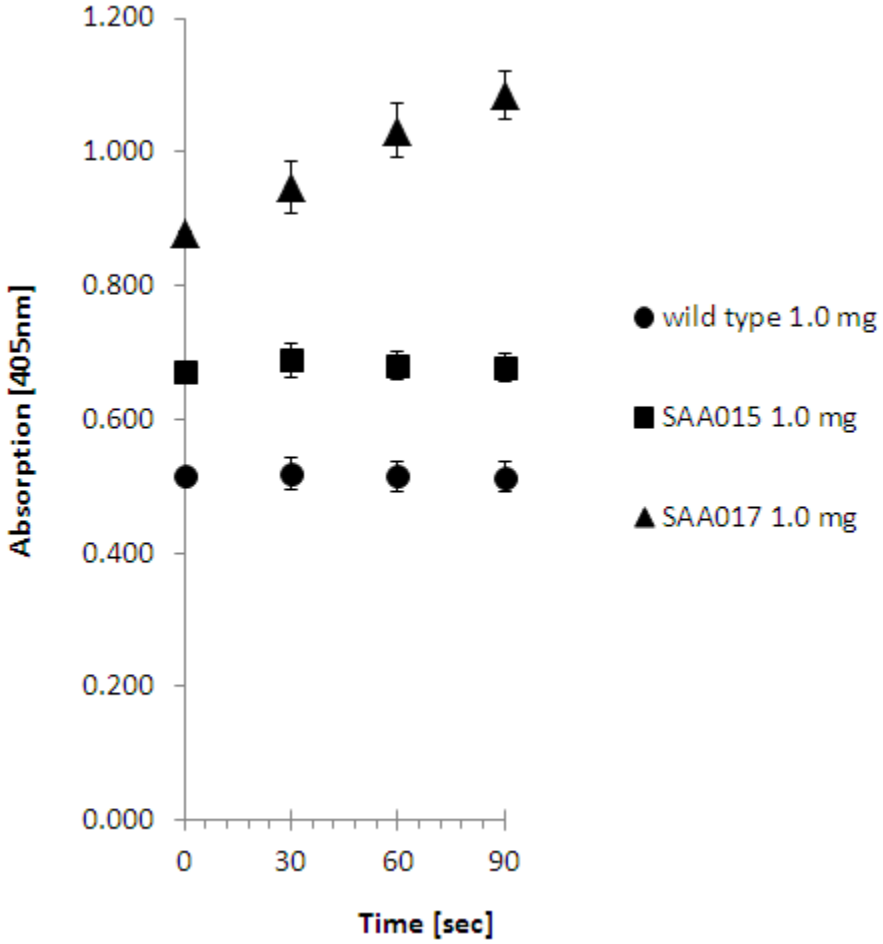
For the calculations on the carbon partitioning we used our measured values of the highest growth rate, which were determined from the exponential growth phase, and the highest rate of lactic acid production, which was determined from the late-exponential growth phase (note that higher values for the partitioning of CO_2 over product *versus* biomass would be obtained, if the actual growth rate during the late-exponential growth phase would be chosen). For the synthesis of 1 mole of biomass and lactic acid 4 and 3 moles of carbon are needed, respectively. Enzyme activities (*i.e.* V_{max} values) are expressed in mmol/gDW/h or in mmol C/gDW/h, thereby taking into account that protein contributes 51 % to the biomass composition of *Synechocystis* (3).

Supplementary Figure 1: Sub-culturing and production stability in engineered mutant strains.



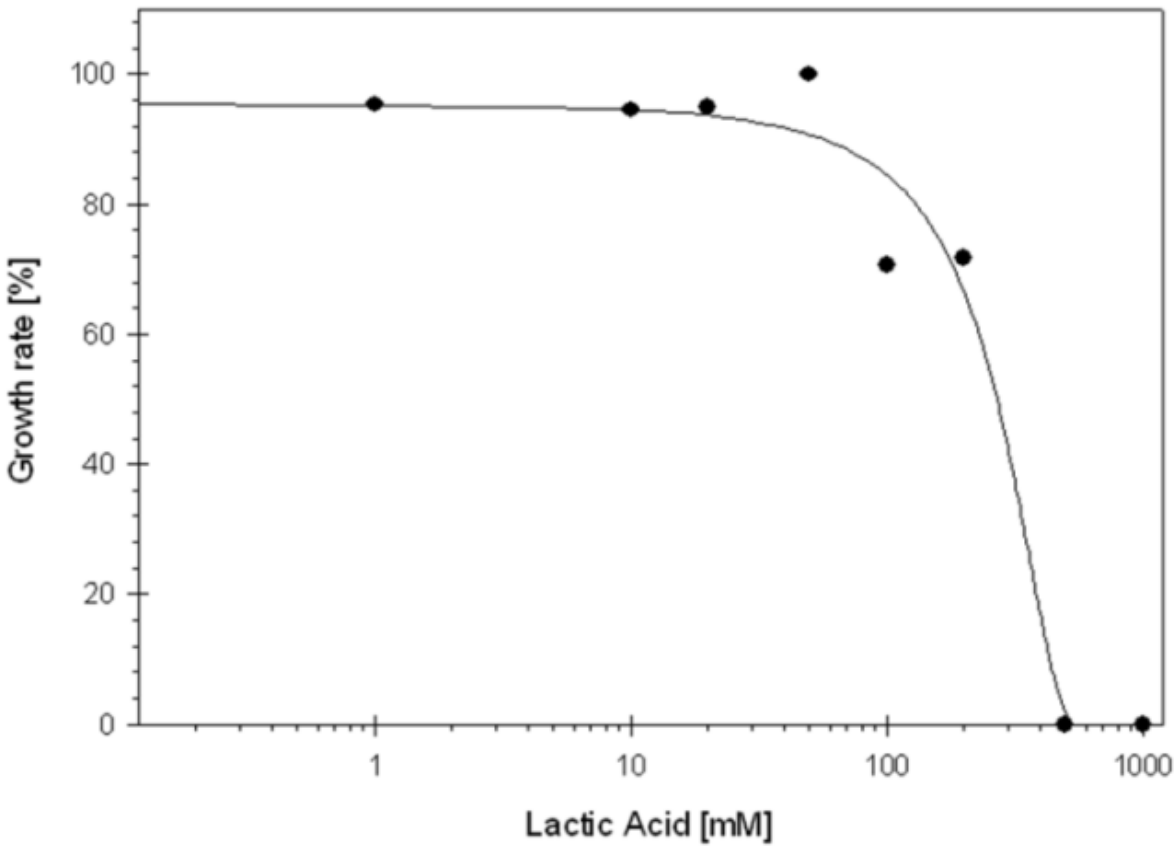
Legend to Supplementary Figure 1: Build-up of the extracellular lactic acid concentration during sub-culturing experiments in batch cultures of engineered *Synechocystis* strains verified the stability of the capacity to produce lactic acid. Cells were grown in BG-11 medium supplemented with 50 mM $NaHCO_3$. Filled symbols represent the OD_{730}; open symbols represent the lactate concentration. SAA015, the strain with integrated *B. subtilis ldh*, is represented by squares; SAA017, the strain with integrated *B. subtilis ldh* and *P. aeruginosa sth*, is represented by triangles. Values are the average of biological replicates; error bars indicate the standard deviation (n=3); if error bars are not visible they are smaller than the data point symbol. (A) First batch cultivation; (B) second subsequent batch cultivation; and (C) third subsequent batch cultivation. The arrows indicate the time point when cells were transferred to fresh medium with a starting OD_{730} of 0.1.

Supplementary Figure 2: Transhydrogenase activity in wild type *Synechocystis*, SAA015 and SAA017.



Legend to Supplementary Figure 2: Activity of the soluble transhydrogenase in crude cell-free extract of wild type *Synechocystis* (circles), SAA015 (squares) and SAA017 (triangles). Only SAA017, the strain with integrated *B. subtilis ldh* and *P. aeruginosa sth*, showed significant activity. Values are the average of biological replicates; error bars indicate the standard deviation (n=3); if error bars are not visible they are smaller than the data point symbol.

Supplementary Figure 3: Lactate toxicity assay.



Supplementary Figure 3: Determining the toxicity of a D/L-lactic acid racemic mixture, added to the growth medium of *Synechocystis*. Using the Sigma Plot Software Package an IC_{50} (50 % growth rate reduction) of 235 mM can be determined.

Supplementary Table 1: Primers used in this study.

Primer	Sequence 5'-3'	Purpose
ecldhA_F	GCGGAATTCGCGGCCGCTTCTAGAGGAG GACTAGCATGAAACTCGCCGTTTATAGC	Fw for the *E. coli ldhA*
ecldhA_R	GCGCTGCAGCGGCCGCTACTAGTATTAAA CCAGTTCGTTCGGGC	Rv for the *E. coli ldhA*
bsldh_F	GCGGAATTCGCGGCCGCTTCTAGAGGAG GACTAGCATGATGAACAAACATGTAAAT AAAGTAGC	Fw for the *B. subtilis ldh*
bsldh_R	GCGCTGCAGCGGCCGCTACTAGTATTAGT TGACTTTTTGTTCTGCAAAATG	Rv for the *B. subtilis ldh*
llldh_F	GCGGAATTCGCGGCCGCTTCTAGAGGAG GACTAGCATGGCTGATAAACAACGTAAA AAAG	Fw for the *L. lactis ldh*
lldh_R_II	GCGCTGCAGCGGCCGCTACTAGTATTAGT TTTTAACTGCGGAAGC	Rv for the *L. lactis ldh*
pasth_F	AATGAATTCGCGGCCGCTTCTAGAGATGG CTGTCTACAACTACGACGTG	Fw for the *P. aeruginosa sth*
pasth_R_II	AATCTGCAGCGGCCGCTACTAGTATCAAA AAAGCCGGTTGAGGC	Rv for the *P. aeruginosa sth*
avsth_F	AATGAATTCGCGGCCGCTTCTAGAGGAG GACTAGCATGGCTGTATATAACTACGATG TGGTG	Fw for the *A. vinelandii sth*
avsth_R	AATCTGCAGCGGCCGCTACTAGTATCAAA AAAGCCGATTGAGACC	Rv for the *A. vinelandii sth*
SEQtrc_F	GACGTAATACGACTCACTATAGGGCG	Fw for verification in the P_{trc} plasmid
SEQtrc_R	AGGTTTCCCGACTGGAAAGC	Rv for verification in the P_{trc} plasmid
V_R	TGCCACCTGACGTCTAAGAA	Fw for verification of BioBricks
V_F	ATTACCGCCTTTGAGTGAGC	Rv for verification of BioBricks
H1_SacI_F	AAATGAGCTCCCTTTGACAACAATGTGGC	Fw for the Hom1 region

	CT	
H1_SacII_R	AAAACCGCGGCCATATAAATCCCCGCCA CT	Rv for the Hom1 region
H2_Xho_F	AAATCTCGAGAGACCAAGCCCAATTTCGT TTG	Fw for the Hom2 region
H2_Kpn_R	AAATGGTACCGCTAAACCCACCTCTTGCC CAAT	Rv for the Hom2 region
Kan903_F	AAATGTCGACAAAGCCACGTTGTGTCTCA AAATC	Fw for the Kan^R cassette
Kan903_R	AAATGTCGACCGCTGAGGTCTGCCTCGTG AA	Rv for the Kan^R cassette
T7_long	GTAATACGACTCACTATAGGGCGAATTG GG	Fw for verification of inserts in pBSK+
T3_long	AATTAACCCTCACTAAAGGGAACAAAAG CTGG	Rv for verification of inserts in pBSK+
H1_seg_F	TGTCGCCGCTAAGTTAGA	Fw for segregation verification in colony PCR
H2_seg_R	CTGTGGGTAGTAAACTGGC	Rv for segregation verification in colony PCR

1. **Emlyn-Jones D., M. K. Ashby, and C. W. Mullineaux**. 1999. A gene required for the regulation of photosynthetic light harvesting in the cyanobacterium Synechocystis 6803. Mol. Microbiol. **33**:1050–1058.
2. **Nogales J., S. Gudmundsson, E. M. Knight, B. O. Palsson, and I. Thiele**. 2012. Detailing the Optimality of Photosynthesis in Cyanobacteria Through Systems Biology Analysis. PNAS.
3. **Shastri A. A., and J. A. Morgan**. 2005. Flux balance analysis of photoautotrophic metabolism. Biotechnol. Prog. **21**:1617–1626.