SUPPLEMENTAL MATERIAL

Chirality matters: Synthesis and consumption of the D-enantiomer of lactic acid by *Synechocystis* sp. PCC 6803

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Running Head: D-lactic acid production and consumption of *Synechocystis*

FIGURE S1 Increase of D- (red symbols) and L- (green symbols) lactic acid in solution in BG-11. The increase can be attributed to evaporation of water, which is approx. 1 % per day, calculated from a 7 day average shown here. Furthermore this serves as contamination control, essentially demonstrating that the lactic acid consumption in the cyanobacterial cultures is not due to a contaminating organism.

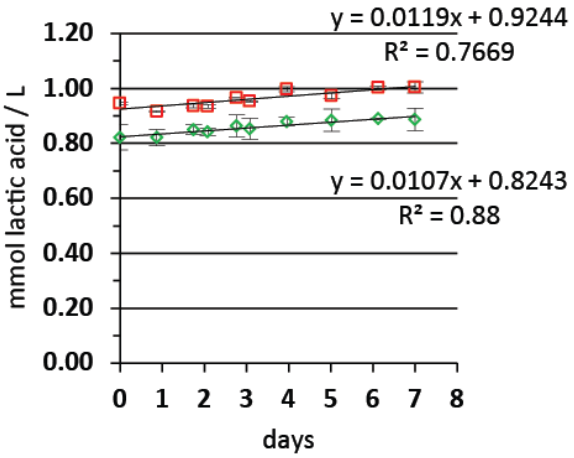

FIGURE S2 (A) Schematic representation of the genetic engineering approach at the neutral site (*slr0168*) and the disruption of the putative D-LDH (*slr1556*) in the genome of *Synechocystis* sp. PCC 6803. Primers used in (B) are indicated. (B) Colony PCR result of relevant strains showing complete segregation at both sites, confirming the gene insertion in ODSYN, DLEU and DLEU/ΔDSYN and the deletion of *slr1556* in ΔDSYN and DLEU/ΔDSYN, respectively.

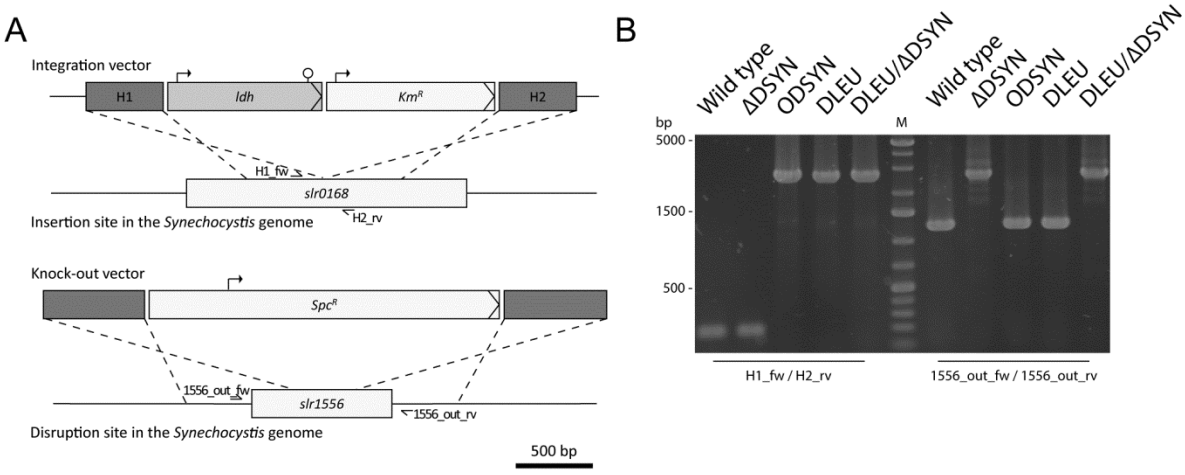

FIGURE S3 Photoautotropic growth and lactic acid production, shown for *Synechocystis* wild type (A), ΔDSYN (B) and ODSYN (C) strains. Closed data points indicate optical density measurements at 730nm [OD], while lactic acid concentration [D-LA] is indicated with the open data points. Error bars indicate the SD (n=3) of biological replicates, except for ODSYN where n=2; if error bars are not visible they are smaller than the data point symbol.

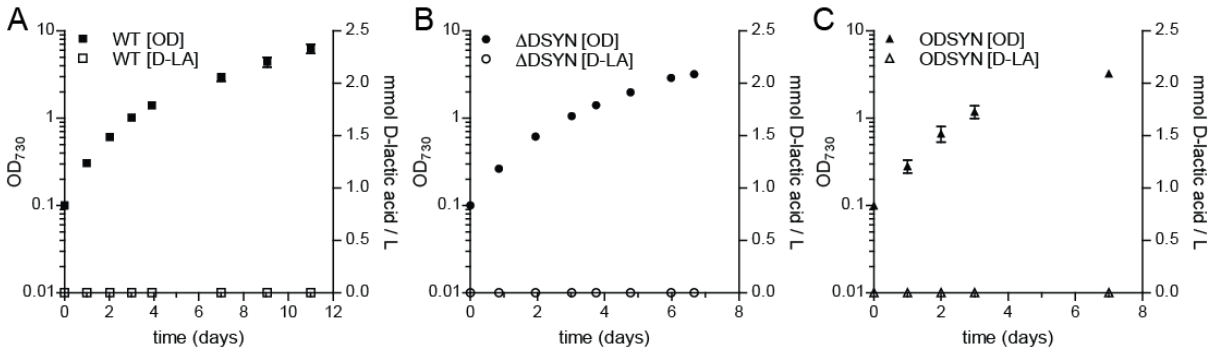

FIGURE S4 Enzymatic activity assay of purified Slr1556. Sigmoidal kinetics with increasing substrate concentrations of (A) D-lactate, (B) glyoxylate, (C) pyruvate, and (D) hydroxypyruvate. Such shapes indicate a cooperating effect. Furthermore a Hill coefficient above one (compare Table 2) suggests positive allosteric regulation.

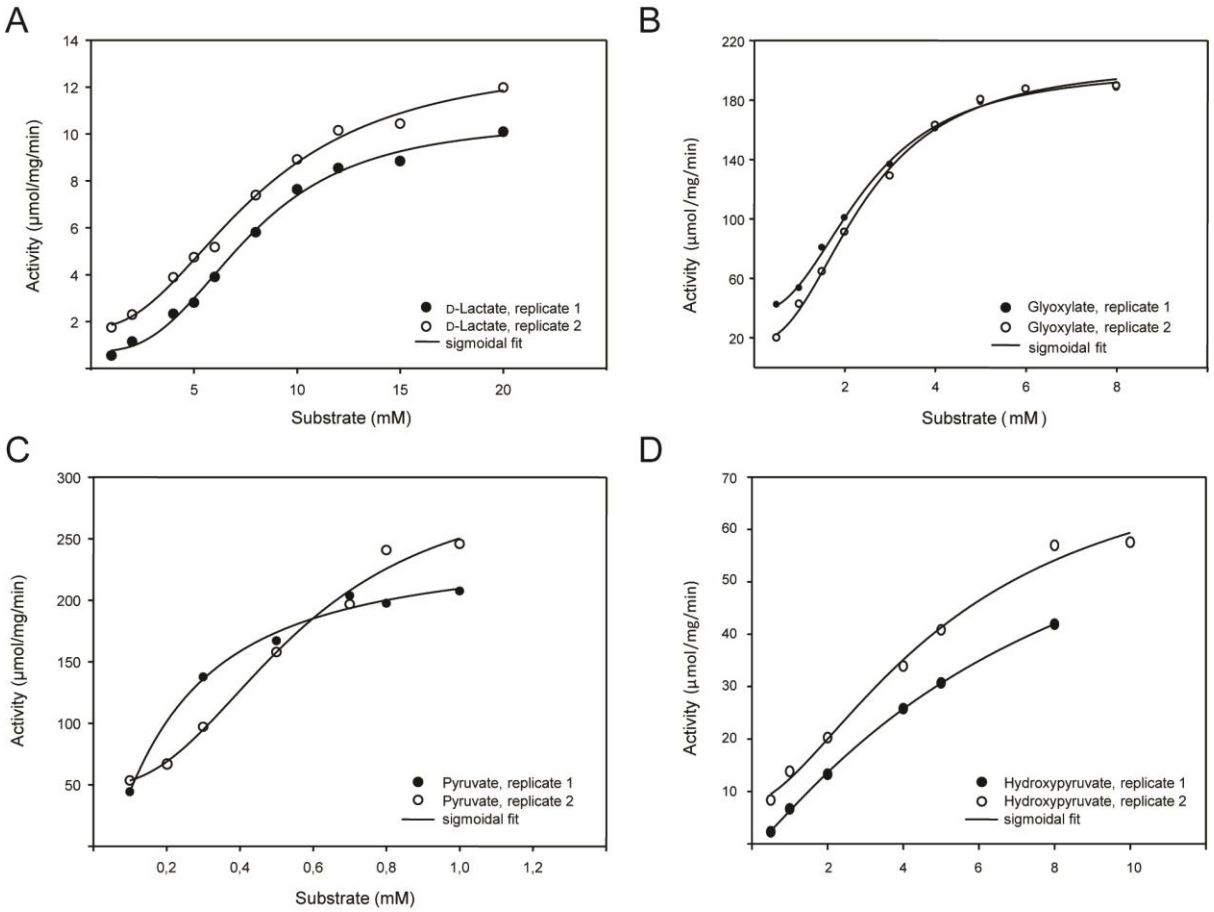

FIGURE S5 D-lactic acid utilization assay of cell extracts of studied through a NAD(P) reduction assay. (A) *Synechocystis* wild type (WT) and (B) *Synechocystis* ΔDSYN strain. NADP shows a certain degree of auto-reduction in both cases.

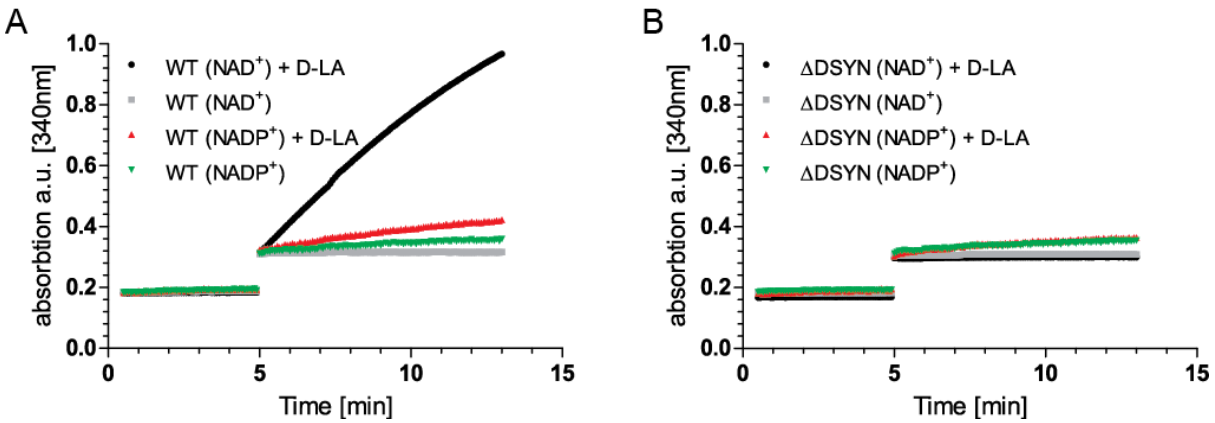

FIGURE S6 (A) Lactic acid detection using HPLC analysis after a prolonged LDH activity assay for wild type (WT) and ΔDSYN, utilizing NADH and NADPH, respectively and pyruvate as starting substrate (STD: standard concentration). Note that chirality cannot be detected by HPLC. (B) Relative NADH- versus NADPH-dependent LDH activity as determined by pyruvate reduction of cell free extracts of WT and ODSYN. Note the three segments of the left Y axis.

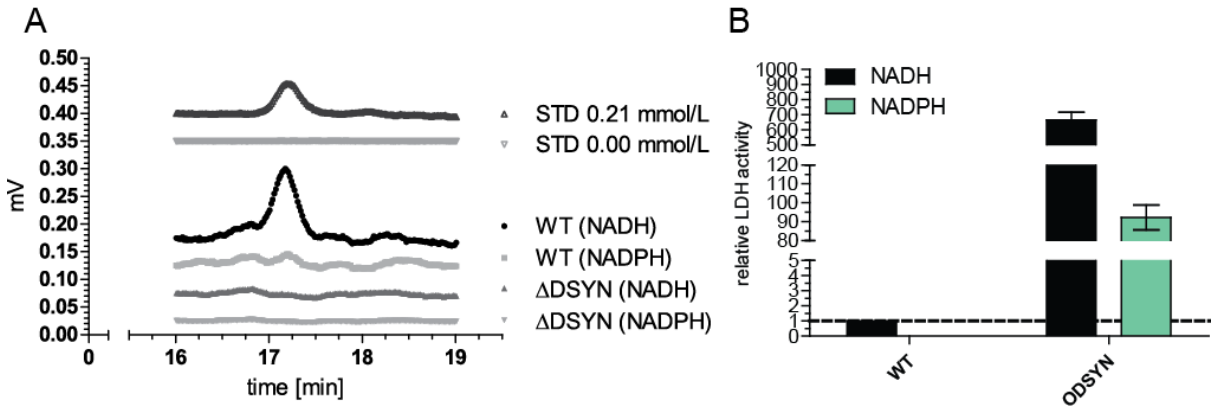

FIGURE S7 Dynamic behaviour of growth with D-lactic acid. Growth rates of wild type supplemented with (D-LA) and without (AT for autotrophic) D-lactic acid. As increase in OD indicates increase in biomass we used moving windows spanning three OD measurement time points to calculate growth rate μ (h^{-1}). Error bars indicate the SD (n=3), if error bars are not visible they are smaller than the data point symbol.