Supplementary File 1

Table S1 Primers used for RT-qPCR in this paper.

Name	**Sequence (5' to 3')**	**Target**
16S-F2	CCACGCCTAGTATCCATCGT	*Synechocystis* 16S
16S-R2	TGTAGCGGTGAAATGCGTAG	*Synechocystis* 16S
TESB3	ACGCTTCTGATCTTAACTTCCTG	*tesB*
TESB4	TCAAAGCTTTTTAATTGTGATTACGCATCAC	*tesB*

Table S2 Comparison of productivities of acetyl-CoA derived biochemicals in cyanobacteria.

Chemical	Cyanobacterial host	Yield ($mg\ L^{-1}$)	Productivity ($mg\ L^{-1}\ day^{-1}$)	Carbon sequestration (mmol carbon $L^{-1}\ day^{-1}$)	References
Fatty acid	*Synechocystis* 6803	197	98.5	5.75	[1]
Acetone	*Synechocystis* 6803	36.0	9.0	0.47	[2]
n-Butanol	*Synechococcus elongates* 7942	404	33.7	1.82	[3]
(*R*)-3-Hydroxybutyrate	*Synechocystis* 6803	533	25.4	0.81	[4]
Fatty alcohol	*Synechocystis* 6803	~2	~0.34	~0.02	[5]
3-Hydroxypropionate	*Synechocystis* 6803	837	139.5	4.65	[6]
(*R*)-3-Hydroxybutyrate	*Synechococcus elongates* 7942	1220	43.6	1.68	[7]
(*R*)-3-Hydroxybutyrate	*Synechocystis* 6803	1845	184.5	5.86	This work

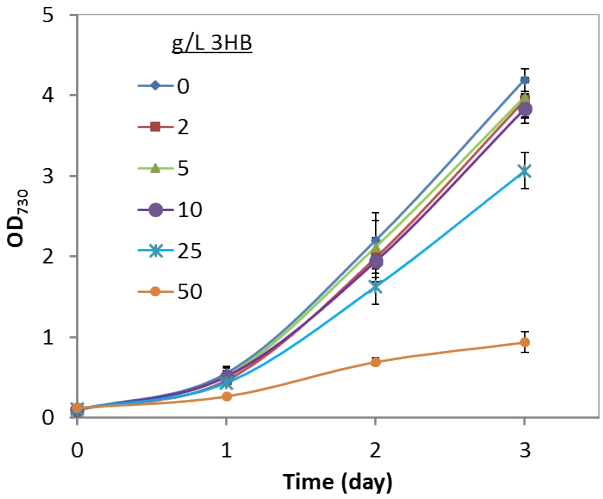

Fig. S1 Growth of *Synechocystis* in BG11 medium supplemented with 10 mM TES buffer, and various concentrations of (±)-3HB. The cultures were placed in a growth chamber aerated with 5% CO_2. Data represent means and standard deviations of three biological replicates. Abbreviations: OD_{730}, optical density at 730 nm; 3HB, 3-hydroxybutyrate.

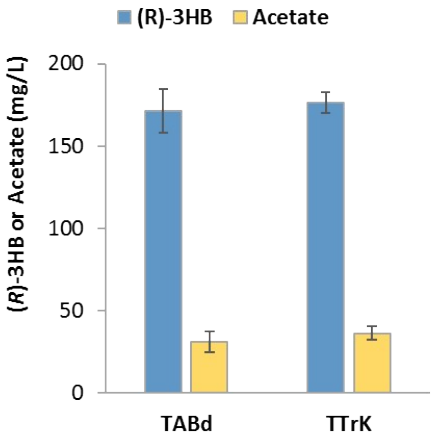

Fig.S2 (*R*)-3HB and acetate production by strains TABd [Cm^R-P_{tac}-*phaA*-*phaB1*, P_{tac}-*tesB*-Kan^R, *ΔphaEC*] and TTrK [Cm^R-P_{tac}-*phaA*-*phaB1*, P_{tac}-*tesB*-T1-Kan^R, *ΔphaEC*] (with an additional *rrnB* T1 terminator downstream *tesB*) in BG11 medium supplemented with 50 mM bicarbonate every day, under a light intensity of 60 µE m^{-2} s^{-1}.

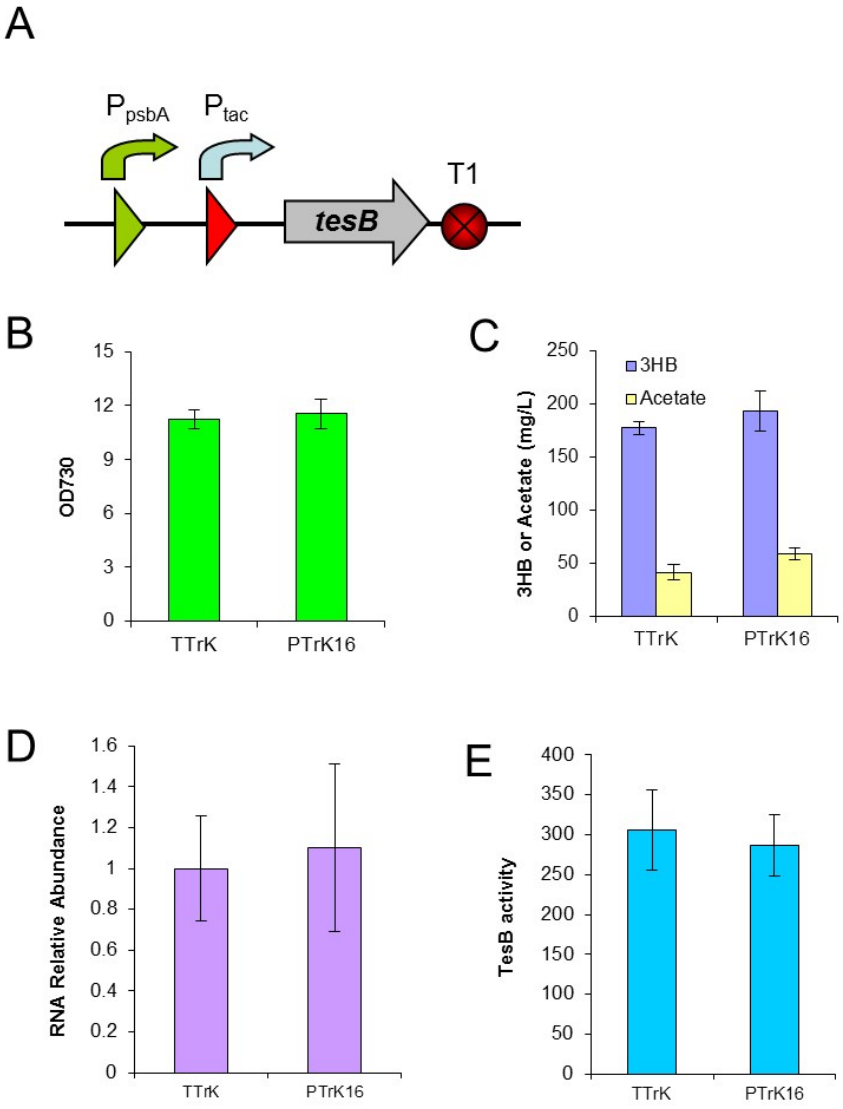

Fig. S3 Characterization of a dual promoter system in *Synechocystis* strain PTrK16. Cells were grown in BG11 medium supplemented with 50 mM bicarbonate every day, under light of 60 µE m^{-2} s^{-1} for 5 days. (A) Schematic representation of the dual promoter system consisting of the P_{psbA} (*psbA2* promoter) and P_{tac} promoters. (B) Cell densities for strains TTrK [Cm^R-P_{tac}-*phaA-phaB1*, P_{tac}-*tesB*-T1-Kan^R, Δ*phchenaEC*] and PTrK16 [Cm^R-P_{tac}-*phaA-phaB1*, P_{psbA12}-P_{tac}-*tesB*-T1-Kan^R, Δ*phaEC*]. (C) Production of (*R*)-3HB and acetate. (D) Relative abundance *tesB* mRNA after photoautotrophic cultivation for 3.5 days. (E) Thioesterase activity analysis for TesB; enzyme activities were given in µmol min^{-1} L^{-1} cell extract.

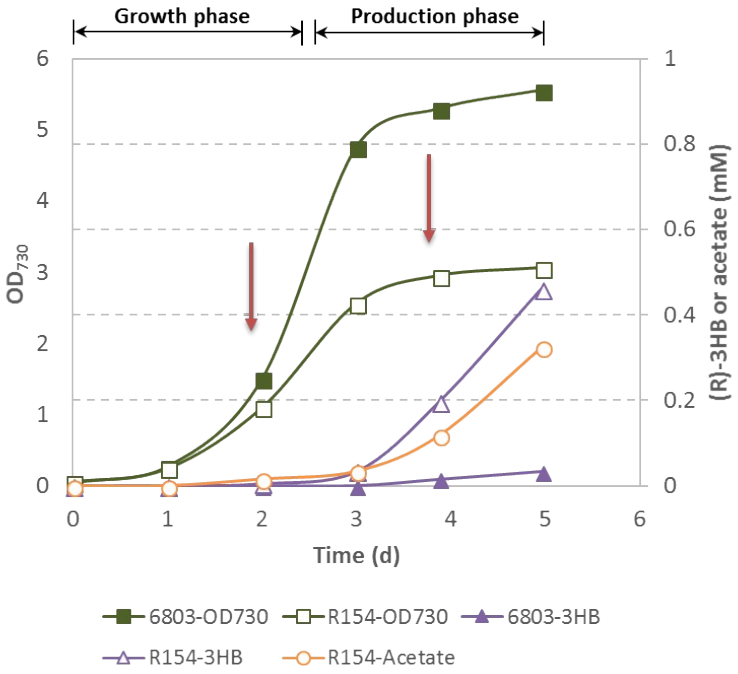

Fig. S4 Growth curve and (*R*)-3HB production curve for *Synechocystis* in the ^{13}C-metabolic flux analysis experiment. Cells were grown in modified BG11 medium supplemented with 10 mM unlabeled glucose and 60 mM $NaH^{13}CO_3$, under light of 60 µE m^{-2} s^{-1}. Red arrows indicate the time points when samples were taken for flux analysis.

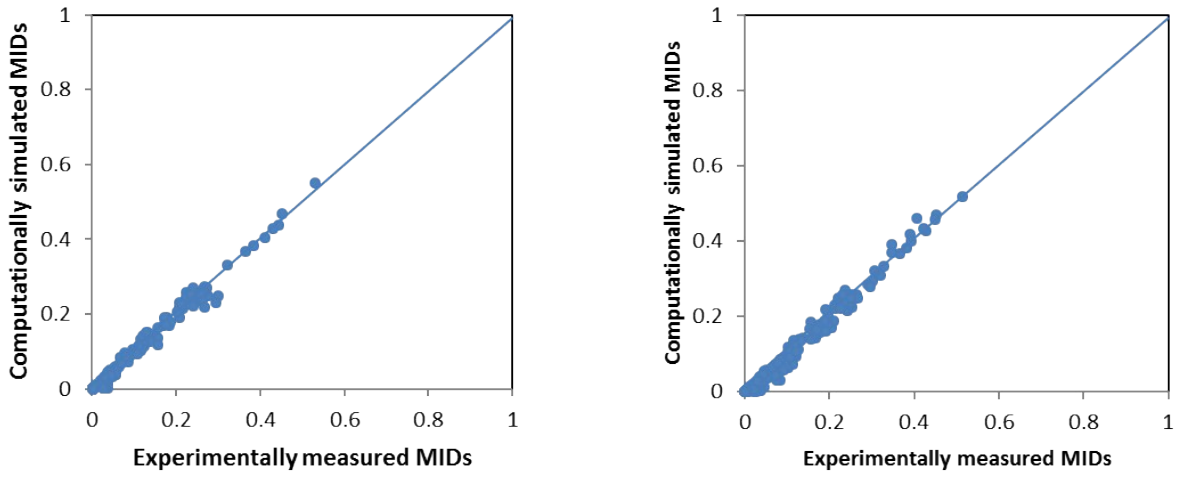

Fig. S5 ^{13}C-MFA model fitting for 3HB producer under the growth phase (left) and production phase (right). 3HB producer were fed by using unlabeled glucose and $NaH^{13}CO_3$.The data shown is the mass isotopomer distributions (MID) of GC-MS fragments for amino acids including ala232, ala260,gly218, gly246, val260, val288, leu274, ile200, ile274, ser288, ser362, ser390, thr376, thr404, phe234, phe302, phe308, phe336, asp302, asp376, asp390, asp418, glu330, glu404, glu432. (Amino acids are represented with their 3-letter abbreviations and the m/z of the unlabeled fragments are represented by following numbers.) The fit between simulated and measured isotopomer distributions is good, with R^2 > 0.985.

References

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