Electronic Supplementary Information

Direct carbon capture for production of high-performance biodegradable plastics by cyanobacterial cell factory

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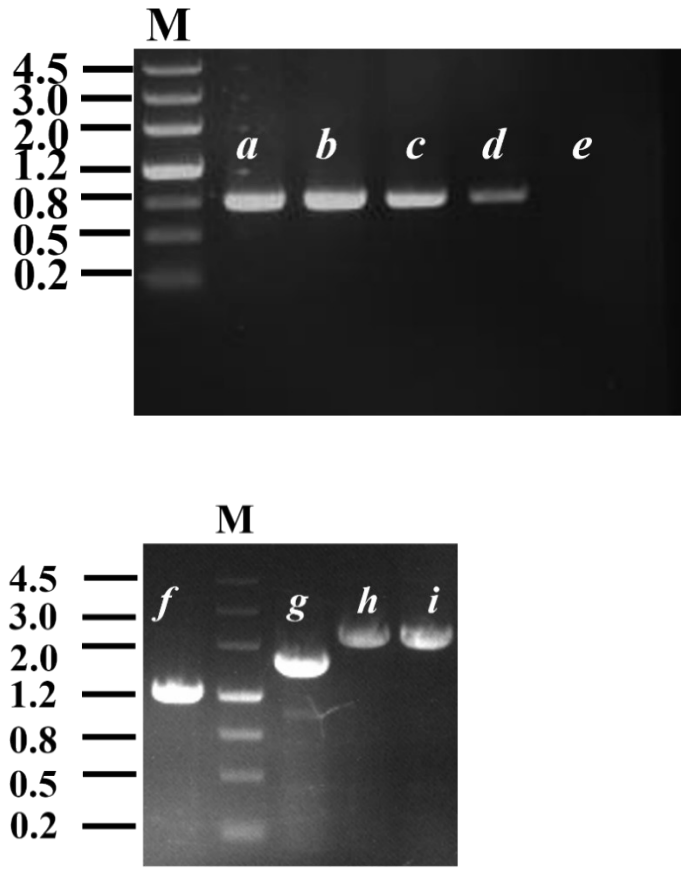
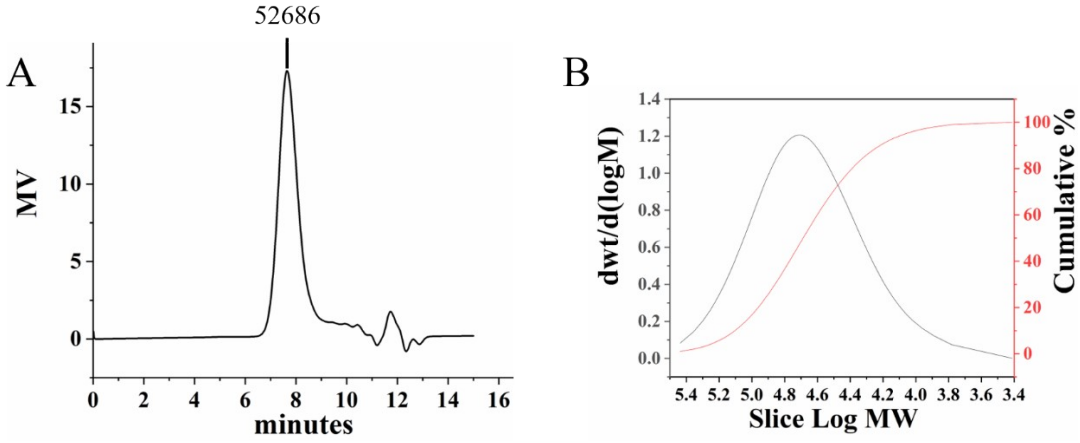


Fig. S1 Colony PCR results with the gene-specific primers confirming the integration of foreign genes in engineered cyanobacteria. The number of “a” to “i" represents the target gene, respectively: *asackA*, *asfabF*, *asfabH*, *asaccC*, wild type, *ldhD*, *pha*, *pct* and *acsA*.



	Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1		32765	62492	52686	108363	173940	1.907246	1.734045	2.783411

Fig. S2 Gel Permeation Chromatography (GPC) analysis of the extracted PLA polymer sample. (A) PLA was analyzed with chloroform system. (B) The relative distribution plots and table.

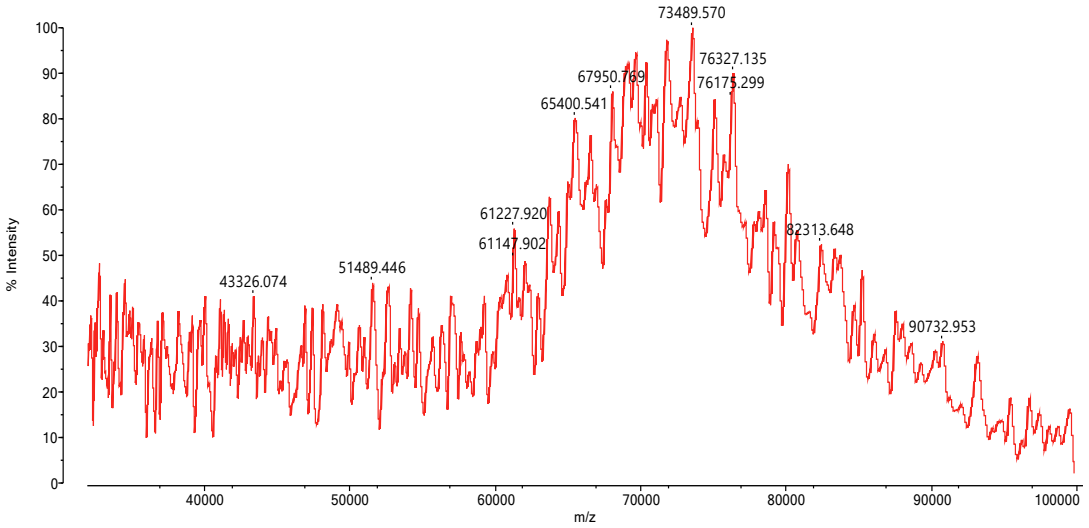


Fig. S3 MALDI-TOF mass spectrum (matrix: DCTB; ionization salt: CF_3CO_2Na; solvent: chloroform) of PLA obtained from the extracted polymer sample.

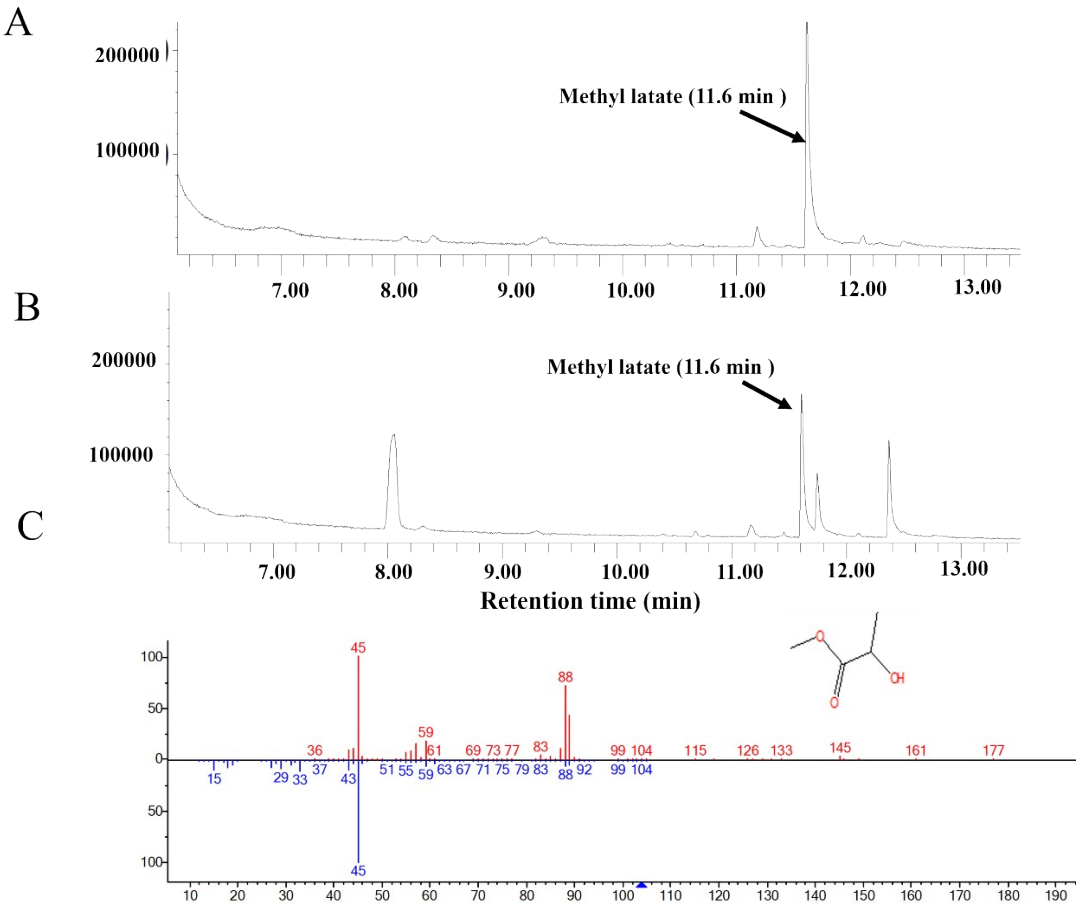


Fig. S4 GC-MS analysis of the methyl lactate (A) The methyl lactate standard substance and (B) culture of the recombinant strain PYLW01. The retention time of methyl lactate was 11.6 min. (C) The mass spectra of the recombinant strain PYLW01 and authentic standard.

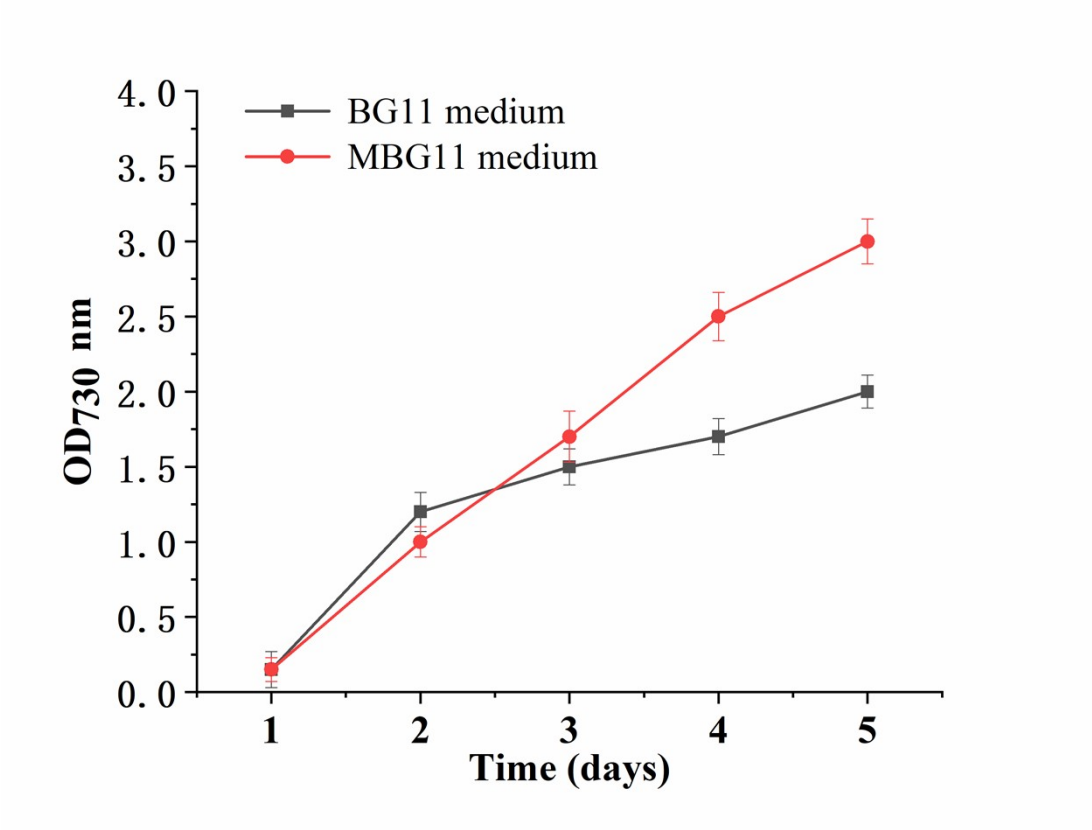


Fig. S5 Effect of medium on the growth of the recombinant PYLW07 strain. The PYLW07 strain was cultured in BG11 medium and MBG11 medium at 30°C with a light intensity of 150μmol m^2s^{-1}. MBG11 medium: BG11 medium enriched with 0.2 g/L $NaCO_3$, 5 g/L $MgSO_4 \cdot 7H2O$, 5 g/L $NaNO_3$, 2 mM KH_2PO_4. Error bars indicate SD (n = 3).

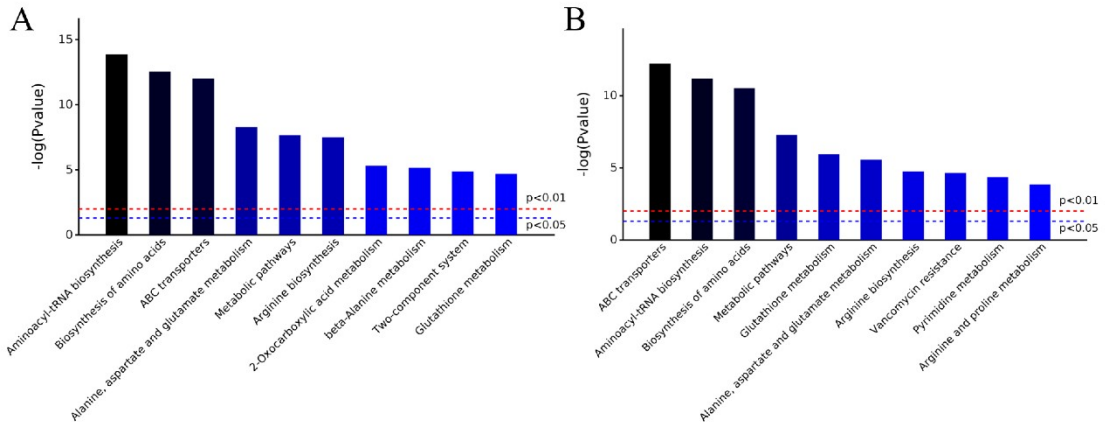


Fig. S6 The analysis of significant differences between different metabolic pathways. (A) The differences between strains T7942 and TPLA. (B) The differences between strains TPLA and TPLASG.

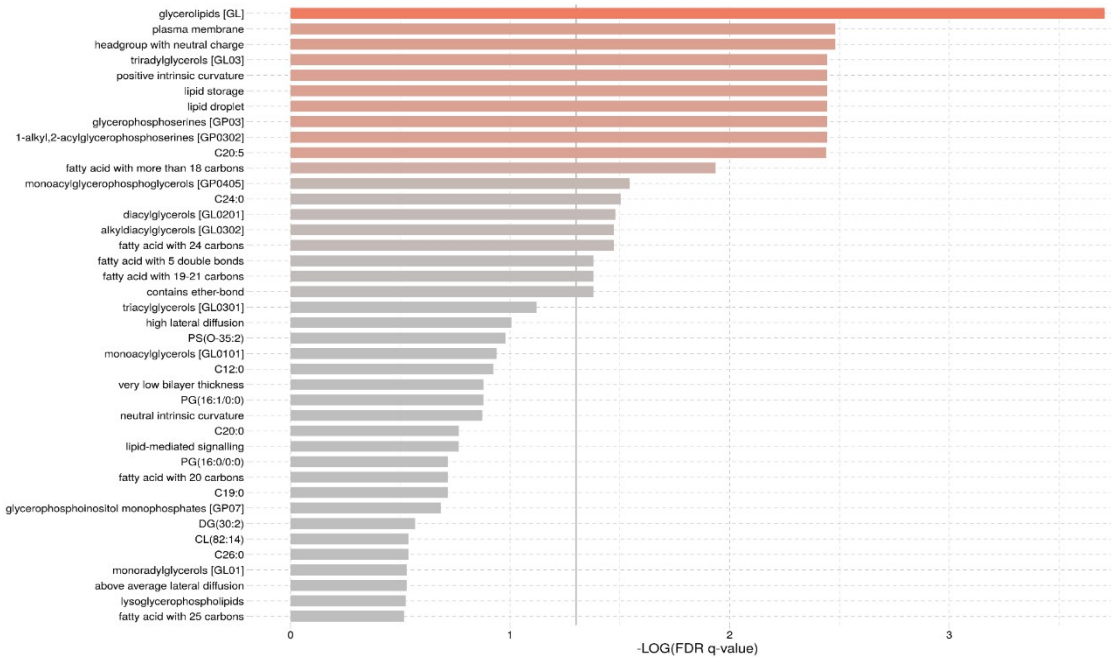


Fig. S7 The enrichment analysis for the lipidome data in TPLASG calculated from the LION website.

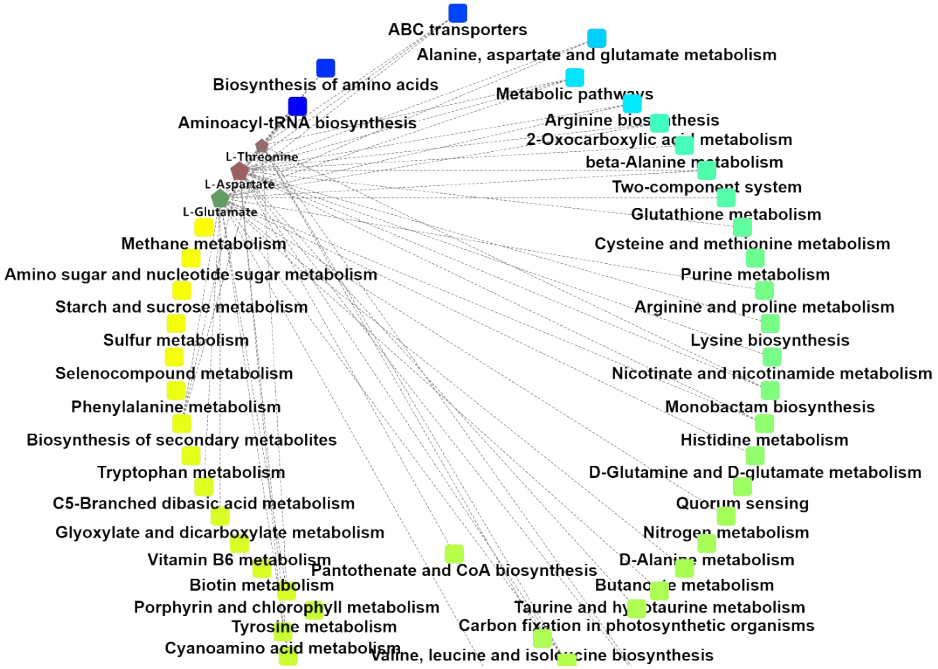


Fig. S8 The graph of protein-protein interaction in strain TPLA.

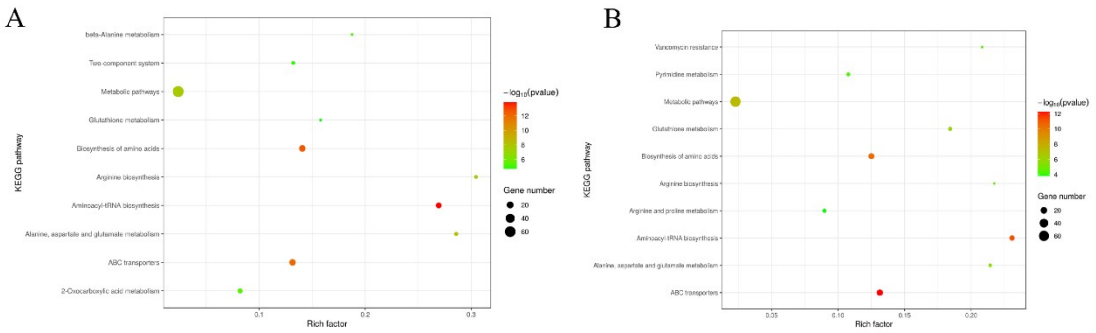


Fig. S9 Distribution of the enriched KEGG pathway. (A) Distribution of the enriched KEGG pathway in strain TPLA compared with strain T7942. (B) Distribution of the enriched KEGG pathway in strain TPLASG compared with strain TPLA.

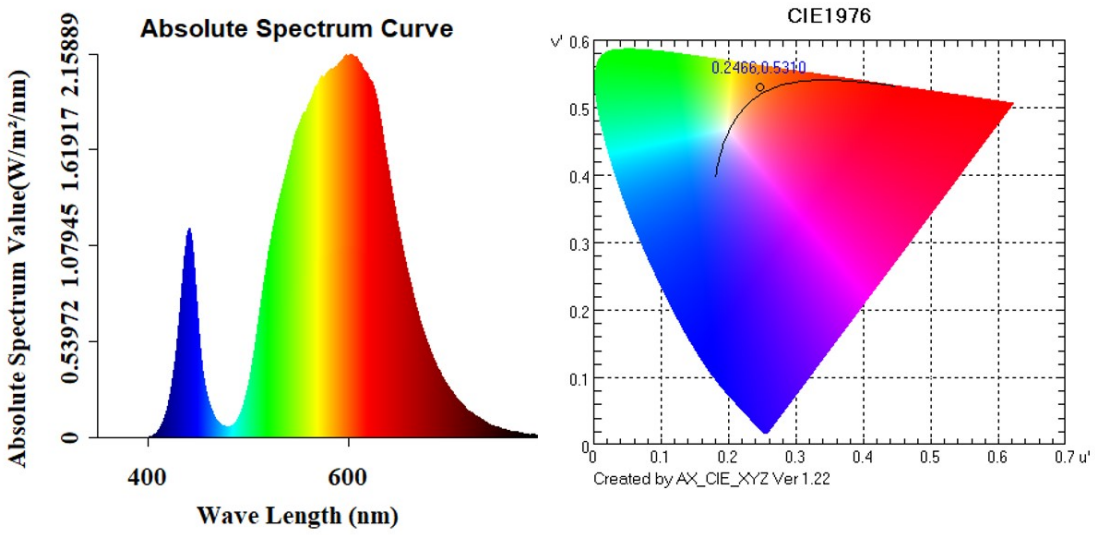


Fig. S10 The absolute spectrum curve data by our photobioreactor.

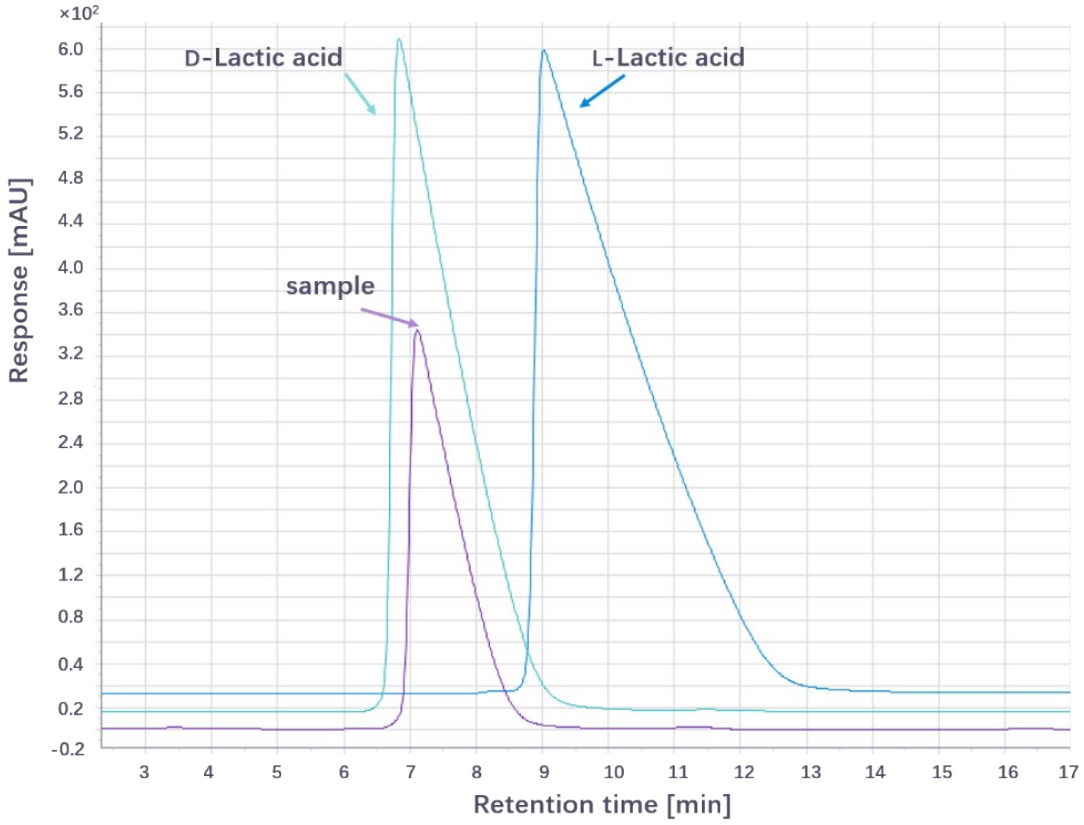


Fig. S11 The analysis of the stereoisomers of lactic acid produced by the engineered cyanobacterial strain PYLW07.

Table S1. Strains used in this study.

Strain	**Description**	**Source**
E. coli DH5	Cloning host	Invitroge
*Synechococcus elongatus*PCC7942	Wild type	ATCC 33912
PWLW01	PCC7942 with *ldhD, pct* and *pha* integrated at NSI	This study
PYLW02	PYLW01 with P_{trc} promoter targeting *pct* and *pha* gene integrated at NSI	This study
PYLW03	PYLW01 with P_{psba} promoter targeting *pct* and *pha* gene integrated at NSI	This study
PYLW04	PYLW01 with P_{cpc560} promoter targeting *pct* and *pha* gene integrated at NSI	This study
PYLW05	PYLW02 with *acsA* integrated at NSIII	This study
PYLW06	PYLW05::P_{psbA}-*asacka-micC*-TrbcL-P_{th}-riboswitch-*hfq*-TrbcL integrated at NSIII	This study
PYLW07	PYLW06::P_{psbA}-*asacka-micC*-TrbcL-P_{psbA}-*asaccC-micC*-TrbcL-P_{psbA}-*asfabF-micC*-TrbcL-P_{psbA}-*asfabH-micC*-TrbcL-P_{th}-riboswitch-*hfq*-TrbcL integrated at NSIII	This study

Table S2. Plasmids used in this study.

Plasmid	**Description**	**Source**
pEASY-Blunt	f1 ori, T7 promoter, KanR and Amp^R	Transgen
PAM2991	ColE1, trc promoter, NSI targeting, $Spec^R$	Stored in lab
ldhD/pAM	pAM2991 contained the *ldhD* gene	This study
ldhD-pct/pAM	pAM2991 contained the *ldhD* and *pct* gene	This study
ldhD-pct-pha/pAM	pAM2991 contained the *ldhD* and *pct* gene	This study
pBA3031M	pBA3031M vector for expressing sRNAs, Kan^R in WT	Dr.TaoSun [1]
asackA/sRNA	pBA3031M::P_{psbA}-*asackA-micC*-TrbcL-P_{th}-riboswitch-*hfq*-TrbcL; Kan^R in WT	This study
asackA-asaccC/sRNA	pBA3031M::P_{psbA}-*asackA-micC*-TrbcL-P_{psbA}-*asaccC-micC*-TrbcL P_{th}-riboswitch-*hfq*-TrbcL; Kan^R in WT	This study
*asackA-asaccC-asfabF//*sRNA	pBA3031M::P_{psbA}-*asackA-micC*-TrbcL-P_{psbA}-*asaccC-micC*-TrbcL-P_{psbA}-*asfabF-micC*-TrbcL-P_{th}-riboswitch-*hfq*-TrbcL; Kan^R in WT	This study
asackA-asaccC-asfabF-asfabH/sRNA	pBA3031M::P_{psbA}-*asackA-micC*-TrbcL-P_{psbA}-*asaccC-micC*-TrbcL-P_{psbA}-*a-fabF-micC*-TrbcL-P_{psbA}-*asfabH-micC*-TrbcL-P_{th}-riboswitch-*hfq*-TrbcL; Kan^R in WT	This study

Table S3. DNA sequences of the primers used in this study.

Name	**Sequence(5'-3')**
ldhD-F	CATGGAATTCATGACTAAAATTTTTGCTTACGCAA
ldhD-R	CATGCTTAAGTTAGCCAACCTTAACGGGAGTTTC
pct-F	CATGCTCGAGGAACCCTCTTTATAAACGCTACC
pct-R	CATGGGATCCGGTAGTTGTTCACCCAGTAGTTC
pha-F	CATGGGATCCGAAGATATCGTGGAACTGGTG
pha-R	CATGGGATCCATCACTTTGCCATCCTCTATC
accC-F	GATCAGGATCTTGTTGAAACGCATTTTCTGTTGGGCCATTGC
accC-R	ATGCGTTTCAACAAGATCCTGATCATGTATTTGTCGATGTTCAGATT
fabF-F	G
fabF-R	ACGCTGGCGTCCGGTTTCAGTCATTTTCTGTTGGGCCATTGC
fabH-F	ATGACTGAAACCGGACGCCAGCGTATGTATTTGTCGATGTTCAGATT
fabH-R	G
	GTTGAGGCTGCCATCACTGCGCATTTTCTGTTGGGCCATTGC
	ATGCGCAGTGATGGCAGCCTCAACATGTATTTGTCGATGTTCAGATT
	G
pAM-F	ATCTGGTTTGACAGCTTATCAT
pAM-R	TCTGTATCAGGCTGAAAATCTTCT
YLW-F	ATCCGCCAAAACAGCCAAG
YLW-	CCGGCCAGCCTCGCAGAG
R	
sRA-F	TGCGAAGTGATCTTCCGT
sRA-R	AGGGACTCTTCTCTACAGGT
acsA-F	AGACCGAGCGTTCTGAACAACGACTGCACGGTGCACCAA
acsA-F	TCGATCGTTGGCTGGCTCATGAATTCCATGGTCTGTTCCTGTG
AcA-F	TTGCAACTACTAACCTTCAATGCA
AcA-R	TCAATTGAAAGACTGTGGGG

Underlining indicates restriction enzyme cleavage sites corresponding to the primer description.

Table S4. Comparison of trace minerals of intracellular extract and the remaining medium after fermentation in BG11 medium and modified BG11 medium.

Sample	Trace minerals (ppm)					
	B	**Mn**	**Zn**	**Mo**	**Cu**	**Co**
IE-BG11(5×)	0.375493876	0.027609927	0.021589774	0.000952725	0.016059076	0.005154796
IE-MBG11(5×)	0.397327113	0.029237651	0.078601708	0.004269637	0.026723837	0.008417892
BG11(50×)	0.372294111	0.011474452	0.010054592	0.006665369	0.001078022	0.000778925
MBG11(50×)	0.387541232	0.024391152	0.041404961	0.024369408	0.001074729	0.001717891

IE=intracellular extract; MBG11= modified BG11 medium.

Table S5. E^+-factors determined for the process of production of polylactic acid cultivated with the photobioreactor.

Energy source	E^+-factor[a] [kg kg^{-1}] × 10^{-3}
Solar light	–0.54
Clean energy	–0.35 ~ 0.11
Fossil fuel	3.67 ~ 5.39 [b] / or 16.4 ~ 21.2 [c]

[a]The E^+-factor furthermore takes energy demand and the resulting CO_2 emission during enzyme production and purification into account[2].

$$E^+ = \frac{\sum m\,(wastes)}{m(products)}\frac{kg}{kg} + \frac{W \times CI}{m(product)}\left[\frac{kWh \times \frac{kg(CO_2)}{kWh}}{kg}\right] \quad (1)$$

Eq. (1). The E^+-factor. W=electrical power used; CI=carbon intensity. i. e. the local average CO_2 emissions caused for the generation of electricity.

[b] The CI data from the Michaja et al[3].

[c] The CI data from OECD average (2015) and European Union average[4].

Clean energy: nuclear, wind, and solar power.

The detailed calculation process: Every $x$mol of polylactic acid monomer (89 g/mol, Mw) generated accompanying $x$mol CO_2 (44 g/mol, Mw) consumption when directly using solar light or the light derived from electricity during eight days of fermentation using the 25 W purple LED. Take European Union for example, still roughly 50% of the electricity is obtained this way, resulting in CO_2 emissions of 315 g per kWh in 2015[4].

Table S6. The recovery rates of polylactic acid (PLA) in different solvents. All data are averages of three independent experiments.

Solvent	Recovery rate of PLA
chloroform	87.7% ± 2.5%
MeTHF	82.3% ± 0.8%
ethylene carbonate	80.3% ± 3.2%
acetone	77.6% ± 1.4%
ethyl acetate	76.7% ± 2.3%

MeTHF:2-methyl tetrahydrofuran

References

1. T. Sun, S. Li, X. Song, G. Pei, J. Diao, J. Cui, M. Shi, L. Chen and W. Zhang, *Biotechnol. Biofuels*, 2018, **11**, 26.

2. F. Tieves, F. Tonin, E. Fernández-Fueyo, J. M. Robbins, B. Bommarius, A. S. Bommarius, M. Alcalde and F. Hollmann, *Tetrahedron*, 2019, **75**, 1311–1314.

3. M. Pehl, A. Arvesen, F. Humpenoder, A. Popp, E. G. Hertwich and G. Luderer, *Nat. Energy*, 2017, **2**, 939–945.

4. International Energy Agency: 'CO_2 emissions from fuel combustion' Agency, https://dx.doi.org/10.1787/co2-data-en, accessed 12.12.2018.