Supplementary materials

treS gene sequence

1 CAGCCAAAGC TTTCACTTCG CCGTCCTGCC GGGTCTCCTG CGAGGGATCC GCGGGGAAGT

61 CCGCACCCCG GTCGGACTGT TCCCGCAGTG ACTGCTCGGC GGCGCCGGAG CCGTCGGGCC

121 AGCCGCCGGG CGGCGGCGGC AGCACGAACC AGTAGAAGCC GTGACCGGGC AGGGTGAGCA

181 GGTAGGGCAG CTCGCCGATC GCCGGGAACT GCACCCCGCC CATGCACTCC ACCGGGGTGC

241 AGCCTTCGAA GCGGCGCAGG TCCAGCTCCA CCGGCTGCGG GAAGCGCGAG AGGTTGTTGA

301 CGCACAGCAC CCGGTCGTCG CCGTACTCGC GCACGAAGGC CAGCACGCTG GGGTTGGAGG

361 CCGGCAGCTC CACATACGAG CCCAGCCCGA ACACCGGGTG CCGCTTGCGG ATCTCGATCA

421 TCTTGCGGGT CCAGTTCAGC AGCGAGCCGG GGTTGCGCTG CTGGGCCTCC AAGTTGACCG

481 CCTGGTACCC GTAGATCGGG TCCATGATCA CCGGCAGGTA GAGCCGGGCC GGGTCGCAGC

541 GGGAGAAGCC GGCGTTGCGG TCGGGCGTCC ACTGCATCGG GGTGCGCACG CTGTCGCGGT

601 CGCCCAGCCA GATGTTGTCG CCCATGCCGA TCTCGTCGCC GTAGTACAGC ACCGGCGATC

661 CCGGCAGCGA CAGCAGCAGC GCGGTGAACA GCTCCAGCTG GTTGCGGTCG TTGTCCAGCA

721 GCGGGGCCAG CCGGCGCCGG ATGCCGATGT TGGCCTTCAT CCGCGGGTCC TTGGCGTACT

781 CGGCGTACAT GTAGTCCCGC TCTTCGTCGG TGACCATCTC CAGCGTCAGC TCATCGTGGT

841 TGCGCAGGAA GATGCCCCAC TGGCAGTTCT CGGGGATCTT GGGGGTCTGC GCCATGATCT

901 CGGAGATGGG GTAGCGCTGC TCGCGCCGCA CCGCCATGAA GATCCGCGGC ATCACCGGGA

961 AGTGGAAGGC CATGTGGCAC TCGTCCCCGC CGGTGGCCGG GTCGCCGAAG TACTCCACCA

1021 CGTCGGCCGG CCACTGGTTG GCCTCGGCCA GCAGCACCCG GTCCGGGTAC AGCCGGTCCA

1061 CTTCGGCGCG CACCCGCTTC AGATAGGCGT GGGTCTCCGG CAGGTTCTCG CAGTTGGTGC

1121 CCTCGCGGGC GTACAGGTAG GGCACCGCGT CCAACCGGAA CCCGTCGATG CCCAGGTCCA

1181 GCCAGAACCG CAGCACCTCC AGCATCGCCT CCTGCACCGC CGGGTTGTCG TAGTTGAGGT

1241 CCGGCTGGTG GGAGAAGAAG CGGTGCCAGT AGTACTGGCC GCGCACCGGG TCGTAGGTCC

1301 AGTTGGACAC CTCGGTGTCG ACGAAGATGA TCCGCGCGTC CGGGTACTTG TCGTCGGTGT

1361 CGGACCACAT GTAGAAGTCG CCGTACGGCC CGTCGGGGTC CGTGCGGGAC GCCTGGAACC

1421 AGGGGTGCTG GTCGCTGGTG TGGTTCATCA CCAGGTCGGC GATGACGCGG ATGCCCCGCC

1481 GGTGCGCCTC ATCGACCAGC TCCACGAAGT CGCCCAGATC GCCGAACTCC GGCAGGATCT

1541 TGGTGTAGTC GCTGATGTCG TAGCCGCCGT CCCGCAGCGG CGACTGGTAG ATCGGCAGCA

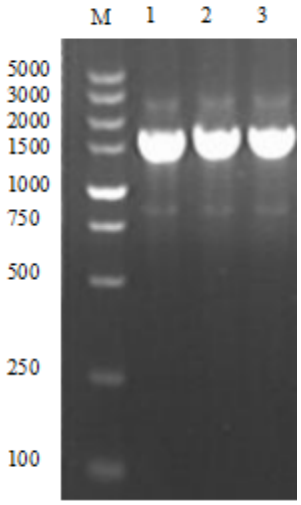
1601 GCCAGATGCA GTCGATGCCC AGCCACTGCA GATAGTCCAG CCGGTTGATG AGGCCGCGCA

1661 GGTCTCCGGT GCCGTCGTCG TTGGAGTCGC TGAACCCGCG CACCAGCACC TCGTAGAAGA

1721 CCGCGTGCTT GTACCAGTAG GGGTCGCGCG GCTTTTCGTG GGTGAAGGTG TCGGGGATGG

1781 GGTCCCCGGT CATCTGATGA

Construction of the engineered trehalose-producing strain

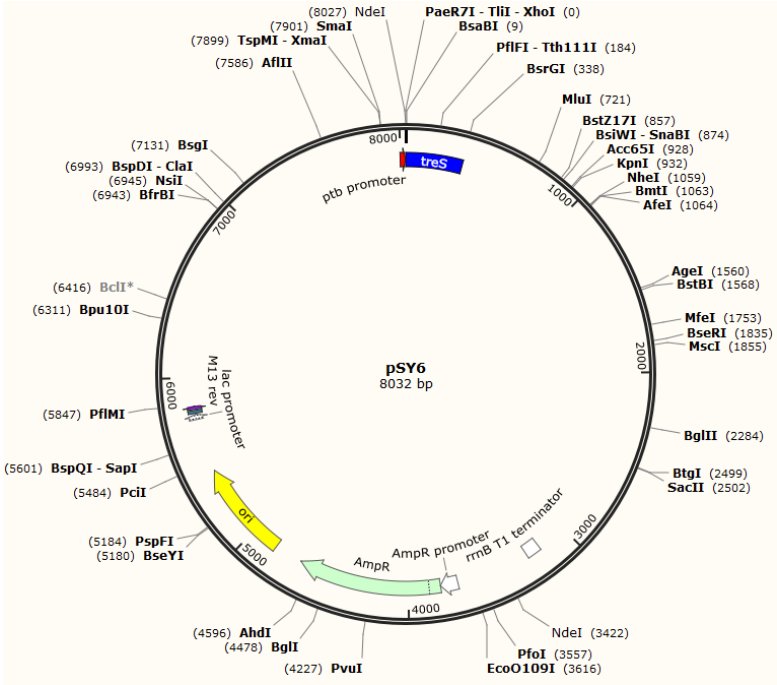


65

66 **Fig. S1-1** The PCR results of *treS* gene amplification (Lane 1, 2, 3 are the three

67 parallel samples).

68



69

70 **Fig. S1-2** Map of the recombinant plasmid harboring the *treS* gene introduced via the

71 *Xho*I and *BsrG*I restriction sites.

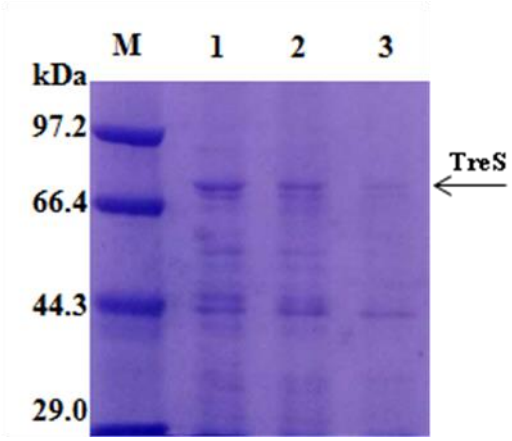


Fig. S1-3 SDS-PAGE analysis. Lane 1, engineered strain with *treS* gene introduced after induction by IPTG for 8 h; Lane 2, engineered strain with *treS* gene introduced after induction by IPTG for 6 h; Lane 3, empty vector pSY6 induced by IPTG for 8 h.

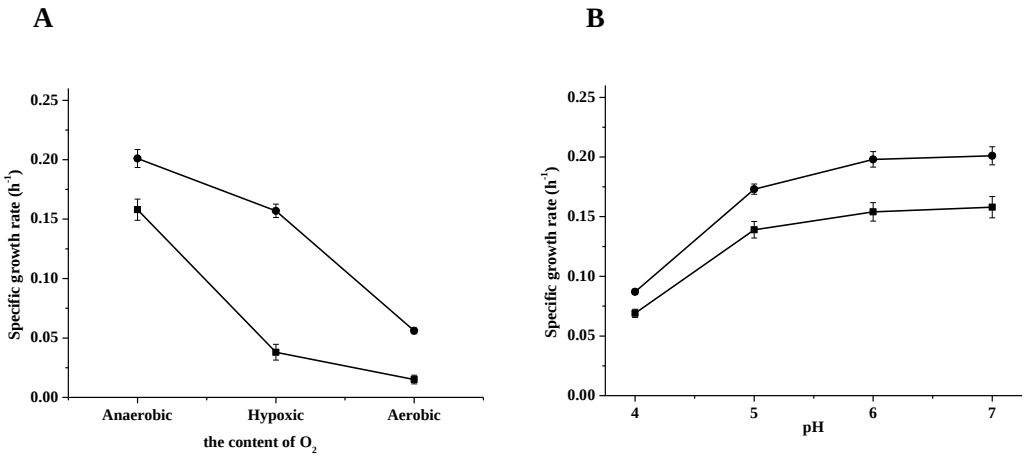
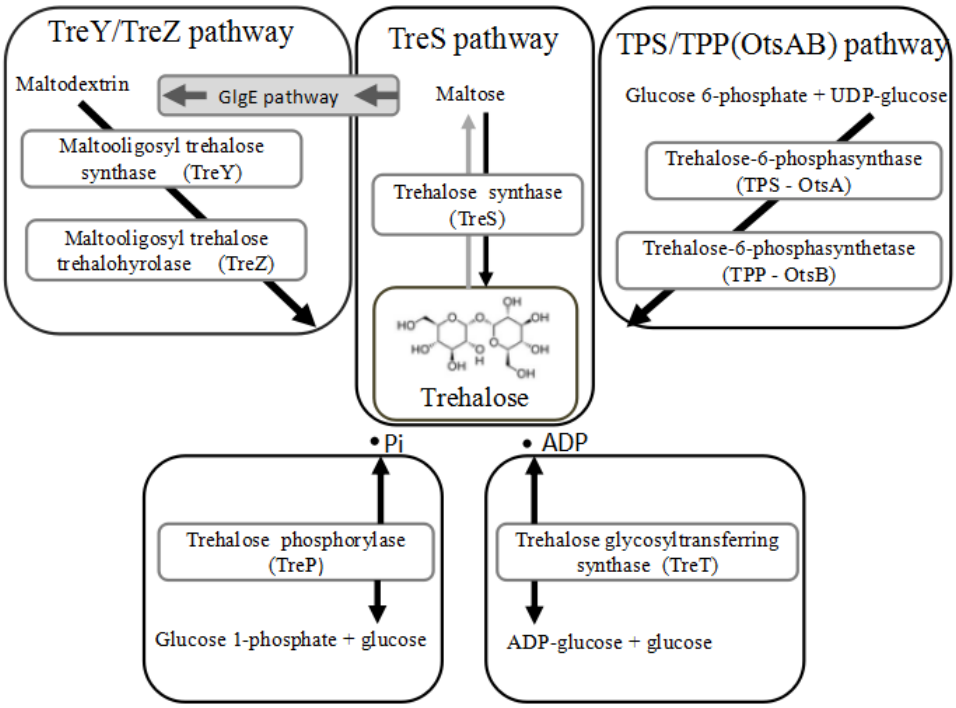


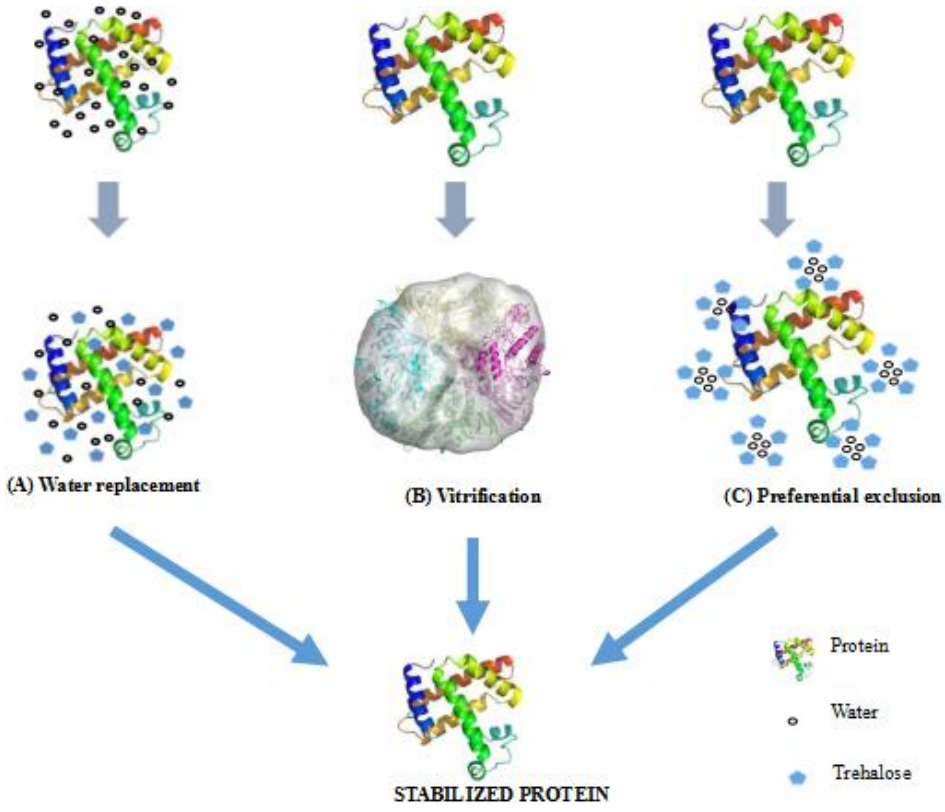
Fig. S2 Specific growth rates of the wild type and the engineered strain in batch fermentations under different contents of O_2 (A) and different pH values (B). Symbols: squares represent ~~wide~~ wild type strain; circles represent engineered strain.



87

88 **Fig. S3** Five trehalose biosynthesis pathways found in different microorganisms.

89



90

91 **Fig. S4** Three hypotheses on how trehalose protects proteins.

92

Table S1 Layout of the 96 wells in AN microplates [a]

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
A	Negative Control	L-Arabinose	N-Acetyl-D-Glucosamine	D-Saccharic Acid	Succinic Acid	D-Galactose	L-Aspartic Acid	L-Proline	D-Alanine	D-Trehalose	D-Mannose	Dulcitol
B	D-Serine	D-Sorbitol	Glycerol	L-Fucose	D-Glucuronic Acid	D-Gluconic Acid	D,L-a-Glycerol Phosphate	D-Xylose	D,L-Lactic acid	Formic Acid	D-Mannitol	L-Glutamic Acid
C	D-Glucose-6-Phosphate	D-Galactonic Acid-g-Lactone	D,L-Malic Acid	D-Ribose	Tween 20	L-Rhamnose	D-Fructose	Acetic Acid	a-D-Glucose	Maltose	D-Melibiose	Thymidine
D	L-Asparagine	D-Aspartic Acid	D-Glucosaminic Acid	1,2-Propanediol	Tween 40	a-Ketoglutaric Acid	a-Ketobutyric Acid	a-Methyl-D-Galactoside	a-D-Lactose	Lactulose	Sucrose	Uridine
E	L-Glutamine	m-Tartaric Acid	D-Glucose-1-Phosphate	D-Fructose-6-Phosphate	Tween 80	a-Hydroxyglutaric Acid-g-Lactone	a-Hydroxybutyric Acid	b-Methyl-D-Glucoside	Adonitol	Maltotriose	2'-Deoxyadenosine	Adenosine
F	Gly-Asp	Citric Acid	m-Inositol	D-Threonine	Fumaric Acid	Bromosuccinic Acid	Propionic Acid	Mucic Acid	Glycolic Acid	Glyoxylic Acid	D-Cellobiose	Inosine
G	Gly-Glu	Tricarballylic Acid	L-Serine	L-Threonine	L-Alanine	Ala-Gly	Acetoacetic Acid	N-Acetyl-D-Mannosamine	Mono-Methyl succinate	Methylpyruvate	D-Malic Acid	L-Malic Acid
H	Gly-Pro	p-Hydroxyphenyl Acetic Acid	m-Hydroxyphenyl Acetic Acid	Tyramine	D-Psicose	L-Lyxose	Glucuronamide	Pyruvic Acid	L-Galactonic Acid-g-Lactone	D-Galacturonic Acid	b-Phenylethylamine	2-Aminoethanol

[a] *C. tyrobutyricum* cell suspensions were inoculated into the 96 wells with pipette and incubated under anaerobic conditions at 37 °C for 24–48 h. The color of broth in each well will ~~turn~~ change into blue from colorless only when the cells can grow in ~~the~~ ~~corresponding~~different carbon source in each well[1,2].

Table S2 Differentially expressed metabolites under the test conditions (Relative amount)

Substances	*Wild type*	*Wild type (O_2)*	*Engineered strain (O_2)*
Propanoic acid	3.037	1.385	3.204
Acetic acid	1.886	1.241	1.745
Butanoic acid	2.293	0.234	1.534
Butanedioic acid	1.358	0.245	1.038
Canavanine	0.561	0.287	0.842
Linolenic acid	0.555	0.104	0.376
Urea	0.418	0.981	0.148
~~d~~D-Mannitol	0.122	0.032	0.042
Fructose	4.907	2.022	4.463
~~d~~D-Galactose	2.547	1.465	2.091
~~d~~D-Glucose	1.202	0.801	2.583
Undecynoic acid	0.141	0.116	0.233
Nonanoic acid	0.229	0.075	0.107
L-Proline	4.169	3.146	4.112
L-Lysine	1.306	0.404	0.334
Glycine	2.211	1.433	2.078
L-Cysteine	0.287	0.216	0.132
Arabitol	2.656	2.459	3.245
Phosphoric acid	0.517	0.378	0.444
Azelaic acid	0.144	0.118	0.085
Tetradecanoic acid	0.301	0.354	0.223
~~d~~D-Mannose	0.395	0.067	0.298
Malic acid	0.322	0.236	0.125
Hexadecanoic acid	2.169	1.485	1.108
Octadecanoic acid	1.265	1.481	1.304
Eicosatrienoic acid	0.211	0.114	0.123
Decanedioic acid	0.223	0.145	0.291
Ethanedioic acid	0.288	0.287	0.399
Pentanoic acid	0.262	0.388	0.231

Benzoic acid	ND	0.069	0.083
Cyclohexanone	ND	0.023	ND
Thiourea	ND	0.019	ND
Canavanin	ND	0.034	0.028
Terephthalic acid	ND	0.011	ND
Heptadecenoic acid	0.021	0.025	ND
Dodecanoic acid	ND	ND	0.018
Stearic acid	ND	ND	0.033
Elaidic acid	ND	ND	0.025
Sebacic acid	ND	0.032	0.038
nonadecenoic acid	ND	0.041	0.029

Note: ND represents not detected.

Table S3 Accumulation of butyric acid in both engineered strain and wild type strain under acidic conditions

strains	g/L [a]	mg per 10^{11} cells
wild type strain	0.09 ± 0.006	0.14 ± 0.01
engineered strain	0.77 ± 0.006	1.21 ± 0.01

[a] The reaction ~~system~~ was carried out in a 500-mL shake~~triangular~~ flask with 100 mL culture medium. The OD_{600} of *C. tyrobutyricum* strain was 0.8 after 12 h, and one OD is equivalent to 0.8×10^9 cells per mL.

REFERNCES

[1] Imam S, Noguera DR, Donohue TJ. 2013. Global insights into energetic and metabolic networks in *Rhodobacter sphaeroides*. BMC Syst Biol 7(1), 89.

[2] Sturino J, Zorych I, Mallick B, Pokusaeva K, Chang YY, Carroll RJ, Bliznuyk N. 2010. Statistical methods for comparative phenomics using high-throughput

phenotype microarrays. Int J Biostatist 6(1), 1-19.