Table S1 Primers used in this study.

Primer	Sequence (5'-3')	Note
Ptef-f	GACGACCTCGCTTAAGCGTGTGAACGCGCTGCCTAGGATG GGCAAGGAGGAGAAG	Cloning promoter P*tef*
Ptef-r	CTAGCTCCAGCCAAGCCCAAAAAATGCTCCTTCAATATCTC GAGTTATTTCTTGGCAGCCTTGGC	Cloning promoter P*tef*
aco-f	CCGCTGCCAAGGCTGCCAAGAAATAACTCGAGATGTTGGC TTCTCGTCAG	Cloning *aco*
aco-r	GTTTGATGATTTCAGTAACGTTAAGTGGATCCCTAGTTCTT AGCGCTCTTGGCC	Cloning *aco*
pfk-f	GTCCGCTGCCAAGGCTGCCAAGAAATAACTCGAGGCCGAA TTGACCATTTTTGTTCC	Cloning *pfk*
pfk-r	GTTTGATGATTTCAGTAACGTTAAGTGGATCCTCATACATA CCCTCTCCTCTCCC	Cloning *pfk*
fba-f	CGCTGCCAAGGCTGCCAAGAAATAACTCGAGGTTCACAAG CGGTTTAAGGC	Cloning *fba*
fba-r	GTTTGATGATTTCAGTAACGTTAAGTGGATCCCAGAAATCA AAAATCGAAATTTTGTTC	Cloning *fba*
U6-f	CTCGCTTAAGCGTGTGAACGCGCTGCCTAGGAGGATCGGT GGAGTGAAGTTCGG	Cloning of U6 promotor
U6-idh-r	GTTTCGTCCTCACGGACTCATCAGTGGTGGGGTACCTATGA TATCCCTTGTTGATGCCCACAGCGCTCGAGGAGGAAAGAA AGAAAAGAAGAGG	Cloning of U6 promotor with *idh* target
gRNA-idh-f	TCGAGCGCTGTGGGCATCAACAAGGGATATCATAGGTACC CCACCACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTC	gRNA5' with *idh* target
gRNA-idh-r	TCCAGATTCGTCAAGCTGTTTGATGATTTCAGTAACGTTAA GTGGATCCGTCCCATTCGCCATGCC	gRNA3'
dcas9-f	CCGCTGCCAAGGCTGCCAAGAAATAACTCGAGGTGAGTTT CAGAGGCAGCAGC	Cloning d*cas9*
dcas9-r	GTTTGATGATTTCAGTAACGTTAAGTGGATCCATGGGTACC TATGATATCAAAAAAATCACACCTTCCTCTTCTTCTTAGGG	Cloning d*cas9*

Table S2 Plasmids and engineered strains used in this study.

Plasmid	Comment
pAN52-1N	Original plasmid
pAN52-P*tef*	Promoter P*tef* replace *gpdA* Promoter and introducing restriction sites including *SnaBI, AflII, AvrII, XhoI, EcoRV, BamHI, NheI, NotI* and *BsiWI* in pAN52-1N
pAN52-P*tef-hph*	*hph* cassettes ligated in pAN52-P*tef*
pAN52-P*tef-cadA-hph*	P*tef-cadA*-T*prc* cassettes ligated in pAN52-P*tef-hph*
pAN52-P*tef-cadA-mttA-hph*	P*tef-mttA*-T*prc* cassettes ligated in pAN52-P*tef-cadA-hph*
pAN52-P*tef-cadA-mtt*A-*mfs*A-*hph*	P*tef-mfsA*-T*prc* cassettes ligated in pAN52-P*tef-cadA-mttA-hph*
pAN52-P*tef-zeo*	*zeo* cassettes ligated in pAN52-P*tef*
pAN52-P*tef-DicadA-zeo*	2-fold *cadA* cassettes ligated in pAN52-P*tef-zeo*
pAN52-P*tef-TricadA-zeo*	3-fold *cadA* cassettes ligated in pAN52-P*tef-zeo*
pAN52-P*tef-QuadcadA-zeo*	4-fold *cadA* cassettes ligated in pAN52-P*tef-zeo*
pAN52-P*tef-aco-zeo*	*aco* cassettes ligated in pAN52-P*tef-zeo*
pAN52-P*tef-neo*	*neo* cassettes ligated in pAN52-P*tef*
pAN52-P*tef-dcas9-U6-gidh-gRNA-neo*	P*tef-dcas9*-T*prc* cassettes and *U6-gidh-gRNA*-T*prc* ligated in pAN52-P*tef-neo*
pAN52-P*tef-bar*	*bar* cassettes ligated in pAN52-P*tef*
pAN52-P*tef-fba-pfk-bar*	P*tef-fba-pfk*-T*prc* cassettes ligated in pAN52-P*tef-bar*
Strain	**Genotype description**
Myceliophthora thermophila ATCC 42464	wild type
IA11	Wild type, overexpressing *cad*A
IA12	Derived from IA11, overexpressing *mtt*A
IA13	Derived from IA12, overexpressing *mfs*A
IA21	Derived from IA13, 2-fold expression of *cadA*
IA22	Derived from IA13, 3-fold expression of *cadA*
IA23	Derived from IA13, 4-fold expression of *cadA*
IA31	Derived from IA13, overexpressing *aco*
IA32	Derived from IA22, knockout of *idh*
IA41	Derived from IA32, overexpressing *pfk* and *fba*

Table S3 Primer sequences used for qPCR analysis in this study.

Primer	Sequence (5'-3')
actin-rt-f	TGCCCATCTACGAGGGTTTC
actin-rt-r	TTGATGTCACGGACAATTTCAC
cadA-rt-f	GCAGTCAGAGCGAGGAGTTT
cadA-rt-r	GGCTTTTCAACGCTTTCGGT
pgk-rt-f	CTCCCCGTCGACTACATCAC
pgk-rt-r	GCCTTGTAGAGCTTGACCGA
sdhB-rt-f	CTTCATGGAGAGGTACGCCC
sdhB-rt-r	TGGCTGAGCTGGAGGTAGAT
pdh-rt-f	GGGCCTTAAGAAACCCGTCA
pdh-rt-r	GGTTGAAGTCACCTGGGAGG
aco-rt-f	CTGAACTCGAGCCCCACATT
aco-rt-r	ACGACGAGTTGGTGCAAGAT
sdhC-rt-f	GGAGTACAGGCAATCCAGGG
sdhC-rt-r	CTCCGAGTTCCACCAGTACG
sdhA-rt-f	GCGTTGAAGGCACAGTGAAC
sdhA-rt-r	GTCCAATGGTAGGACCCGTG
cs-rt-f	ATGTTCAAGCTCGTCAGCCA
cs-rt-r	GTTGGGGTAGGGGTTCTTGG
pepck-rt-f	CCCAAGTCGTGCCCTAATGT
pepck-rt-r	TCCAAGAAGAGAGCGCCAAG
pfk-rt-f	ACTGATCACTGCGCCAAGAA
pfk-rt-r	TACGGGTTCCTCGGACAGAT
fba-rt-f	CAAGTCCGTGTTTGCCAAGG
fba-rt-r	TAGACCCTGGAGGCCTTCTC

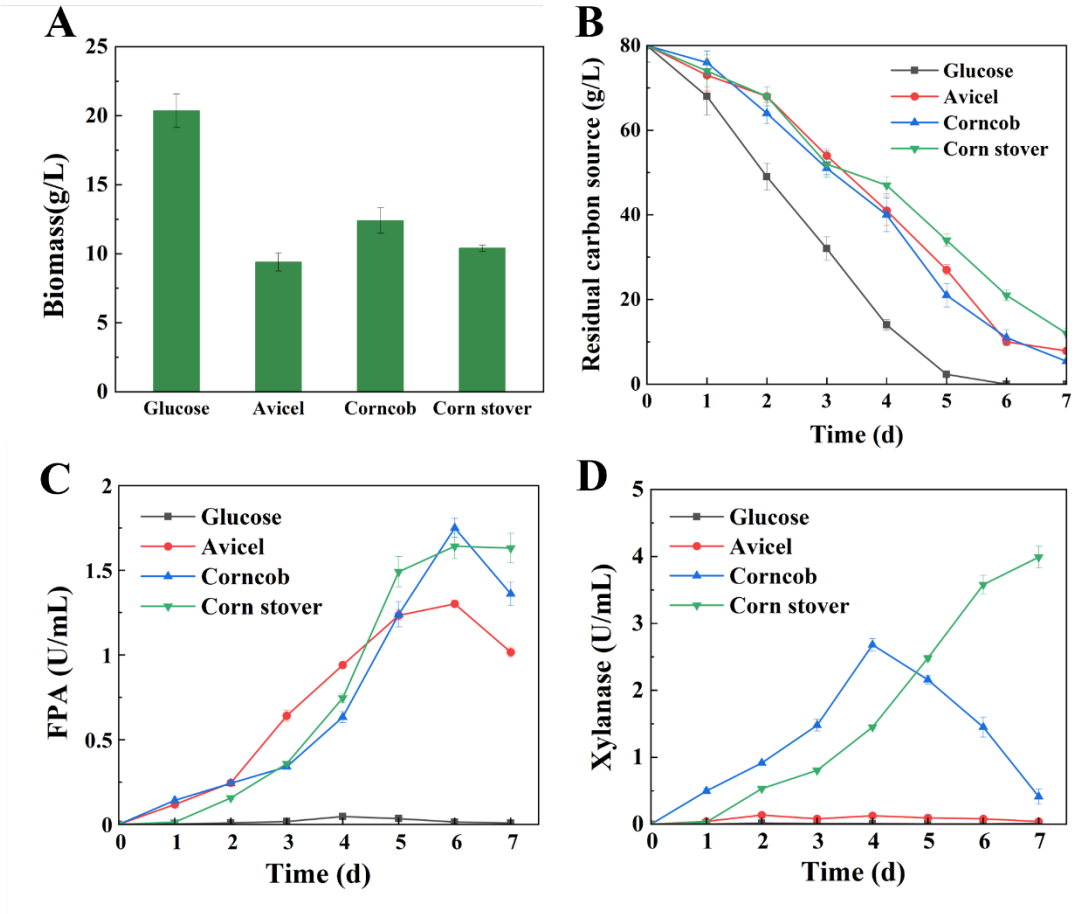


Figure S1 Evaluation of the WT *M. thermophila* as a starting strain for IA production from lignocellulose. (A) Biomass accumulation, (B) residual carbon source concentration, (C) cellulase activity, and (D) xylanase activity of the WT strain after 7-day fermentation under different carbon sources.

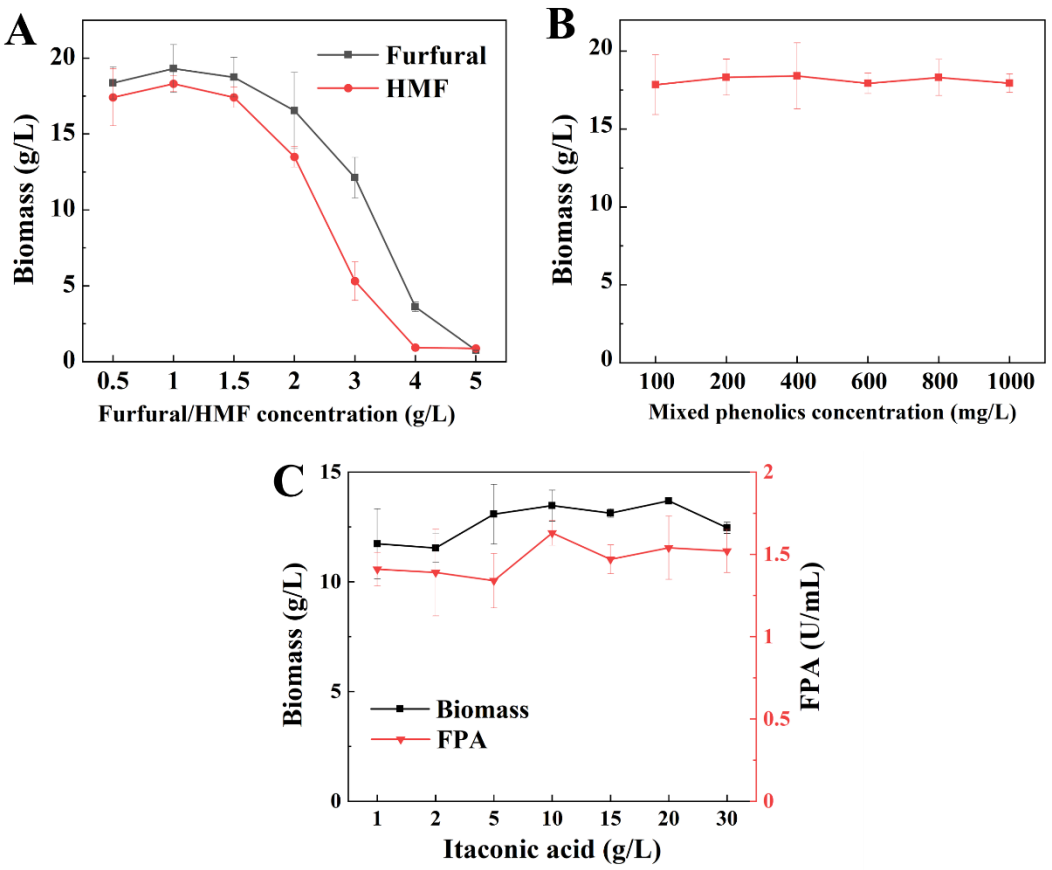


Figure S2 Effect of inhibitors on the growth of *M. thermophila*. Growth of WT strain in media containing different concentrations of (A) Furfural and HMF; (B) Mixed phenolics; and (C) Itaconic acid.

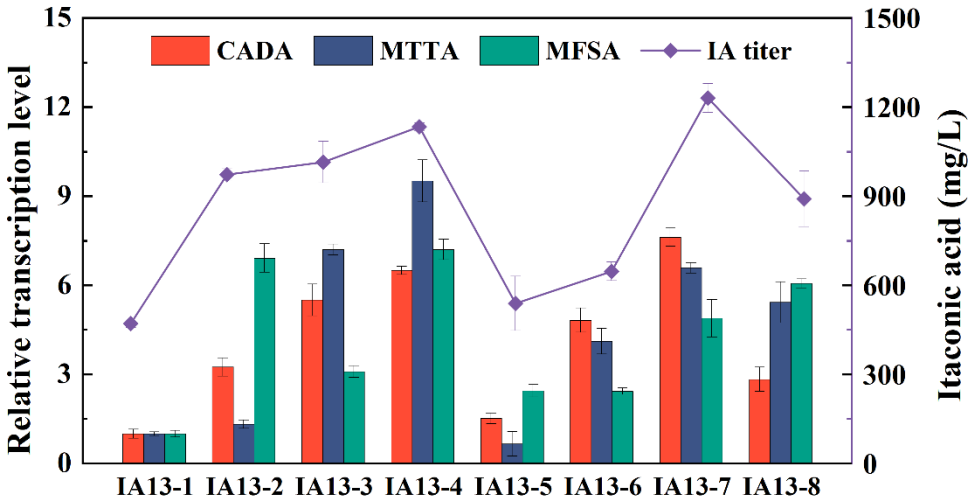


Figure S3 IA titer and the expression levels of the exogenous genes in the IA13 strain

transformant cultured in glucose medium.

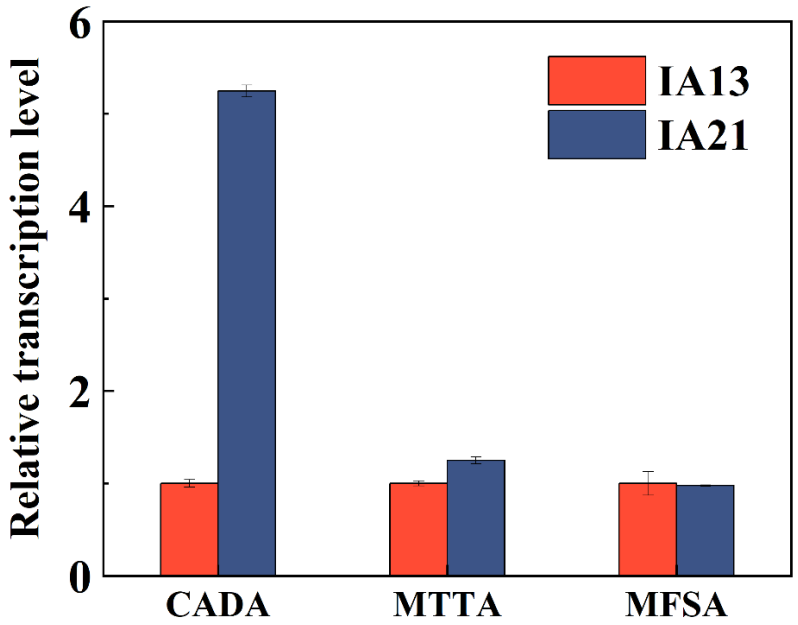


Figure S4 Expression levels of heterologous genes in IA21 and IA13.

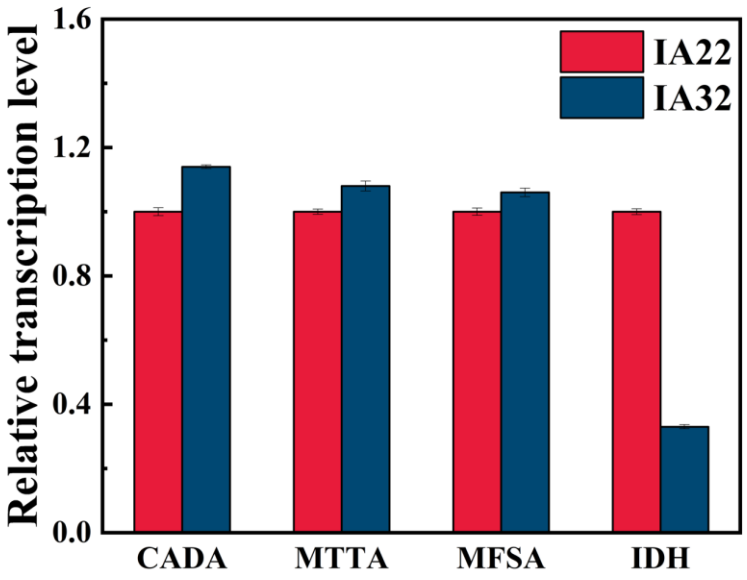


Figure S5 Expression levels of heterologous genes in IA22 and IA32.

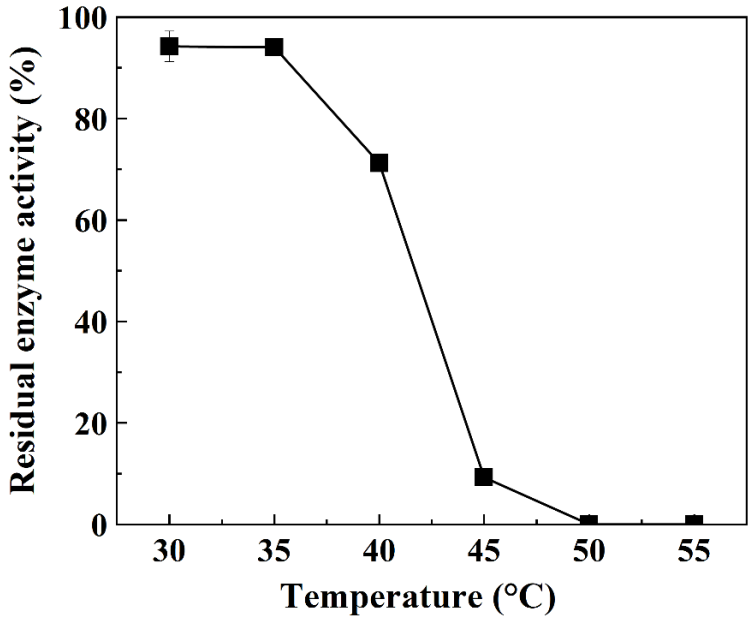


Figure S6 Thermostability of CADA. Crude enzyme extracts were pre-incubated at 30 -55 °C for 30 min, Residual enzyme activity was measured at 40 °C and expressed as a percentage relative to the non-incubated control.

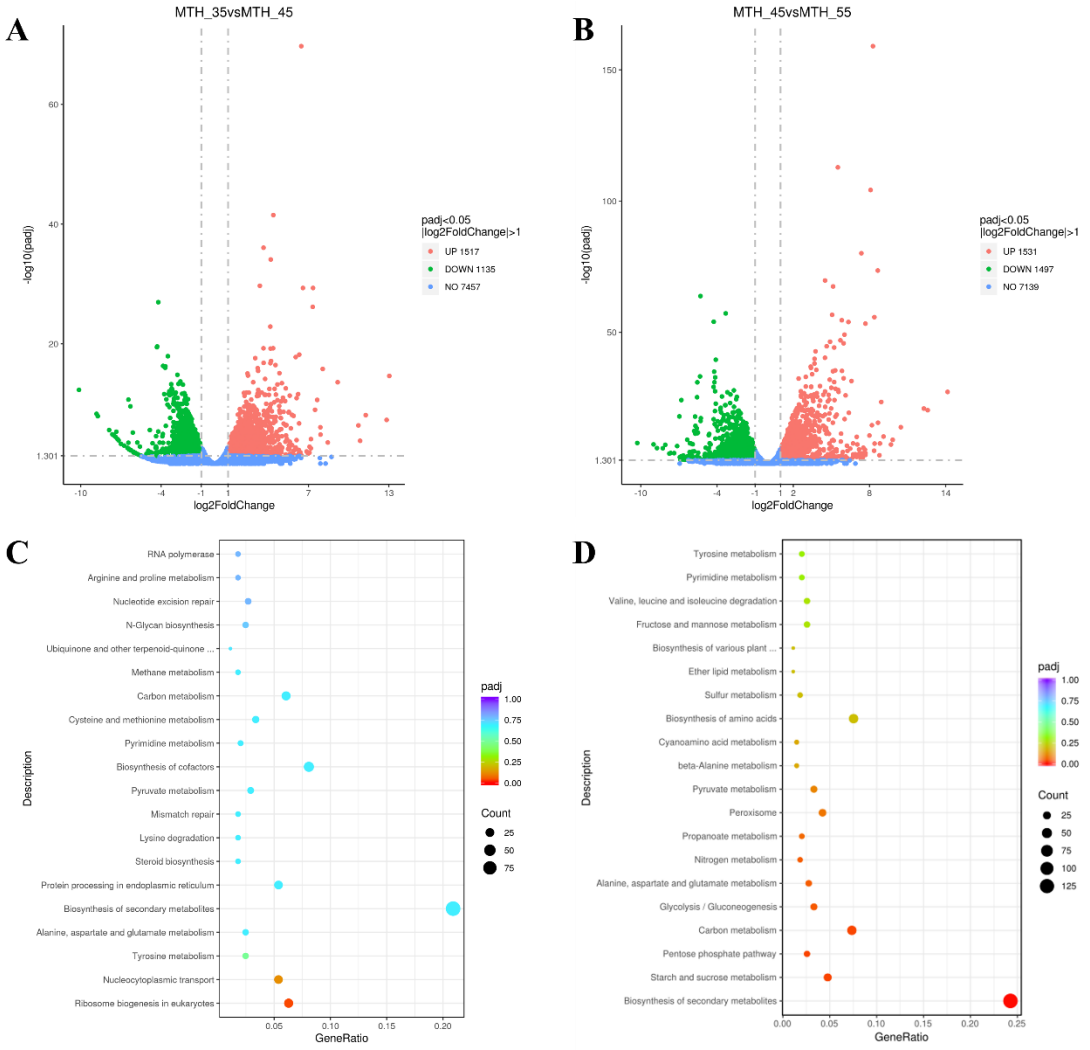


Figure S7 Analysis of differential gene expression in *M. thermophila* strains under different temperature conditions. Volcano plots of differential gene expression between (A) 35°C vs. 45°C and (B) 45°C vs. 55°C. Red points represent significantly upregulated genes, blue points represent significantly downregulated genes, and gray points indicate genes with no significant change. KEGG enrichment analysis of differentially expressed genes between (C) 35°C vs. 45°C and (D) 45°C vs. 55°C.

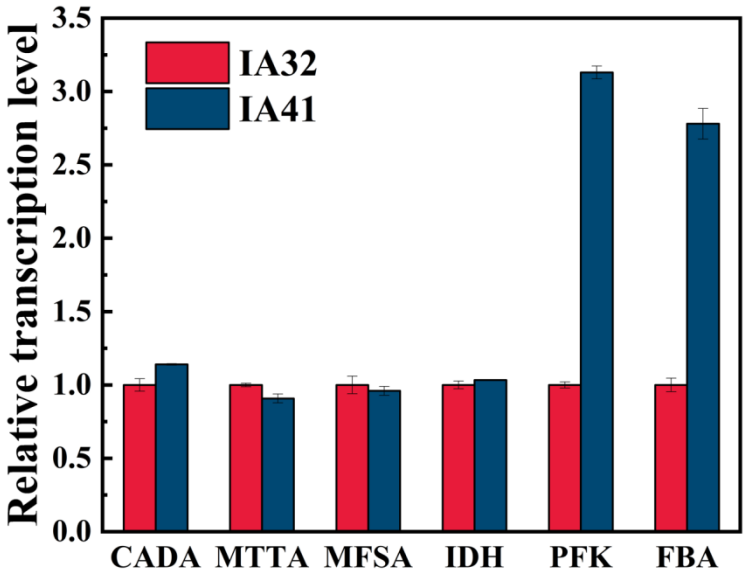


Figure S8 Expression levels of heterologous genes in IA32 and IA41.