Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

High astaxanthin production by *Xanthophyllomyces dendrorhous* strain DW6 from cane molasses using two-stage pH strategies

Dawei Zhou[a], Lixin Yang[a], Fang Guo[a], Wankui Jiang[a],Yujia Jiang[a],

Wenming Zhang[a,b*], Fengxue Xin[a,b*],Min Jiang[a,b],

[a]State Key Laboratory of Materials-Oriented Chemical Engineering,

College of Biotechnology and Pharmaceutical Engineering,

Nanjing Tech University, Nanjing, China

[b]Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM),

Nanjing Tech University, Nanjing, China

* Corresponding authors at: State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Puzhu South Road 30#, Nanjing 211800, P. R. China.

E-mail addresses: bioengine@njtech.edu.cn (M. Jiang)

xinfengxue@njtech.edu.cn (F.X. Xin)

Figure S1

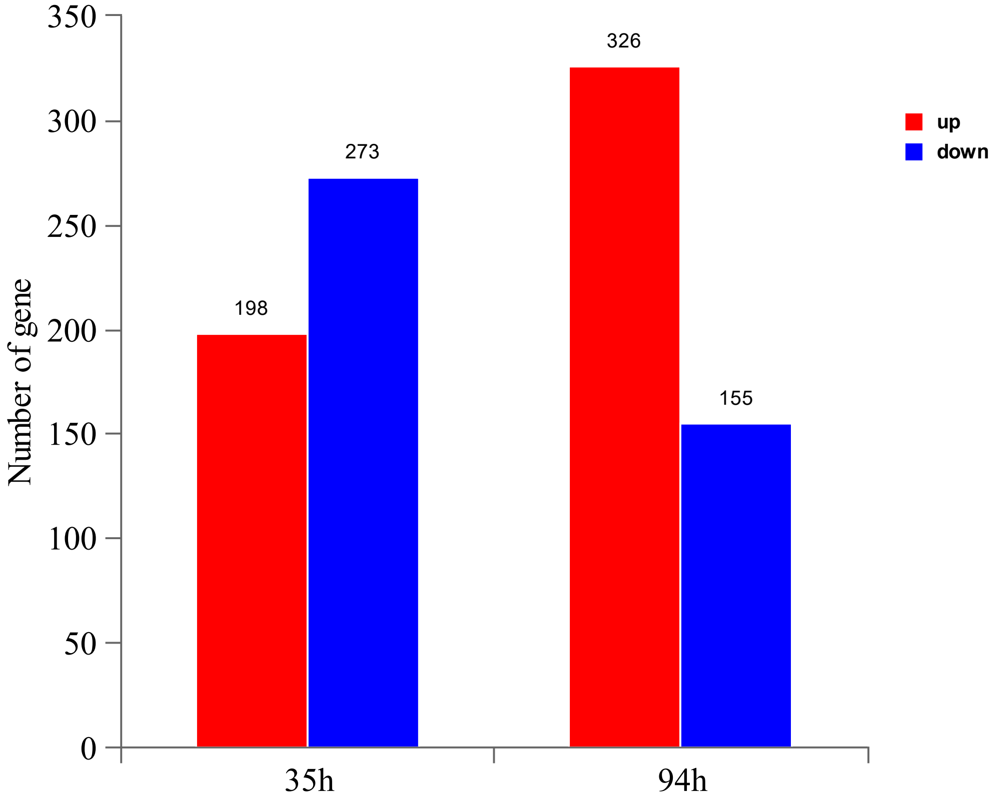
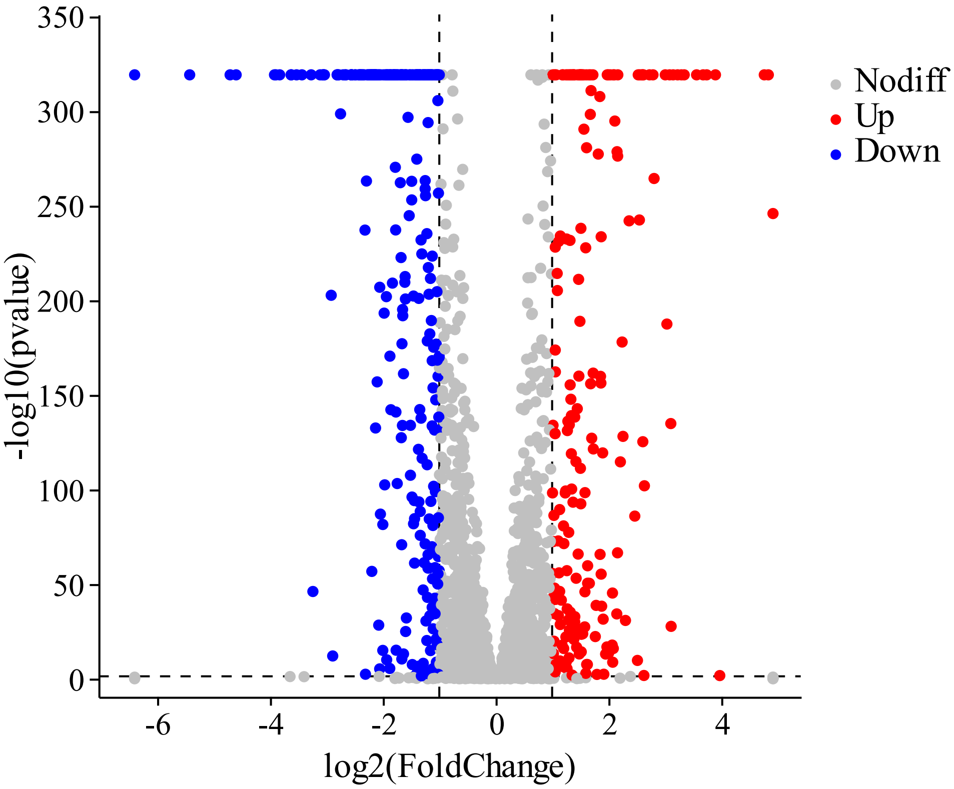

Number of gene
350
300
250
200
150
100
50
0
326
273
198
155
35h
94h
up
down

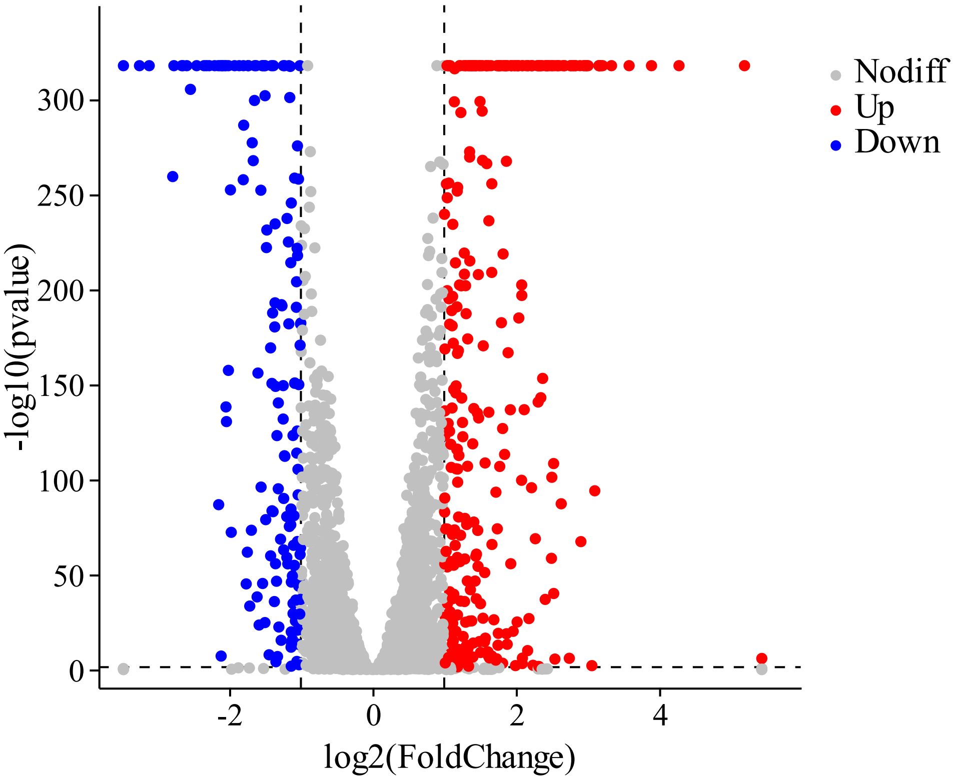
Figure S2

A



350
300
250
200
150
100
50
0
-log10(pvalue)
-6
-4
-2
0
2
4
log2(FoldChange)
Nodiff
Up
Down

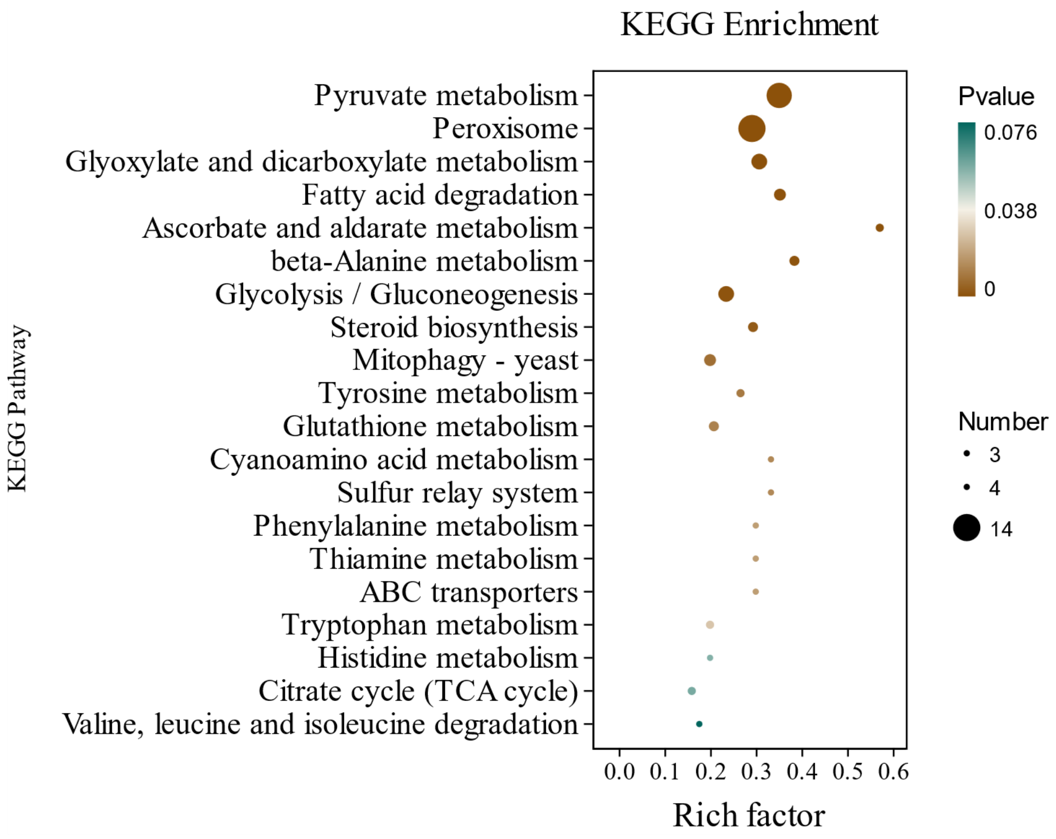
B



300
250
200
150
100
50
0
-log10(pvalue)
-2
0
2
4
log2(FoldChange)
Nodiff
Up
Down

Figure S3

A


KEGG Enrichment
KEGG Pathway
Pyruvate metabolism
Peroxisome
Glyoxylate and dicarboxylate metabolism
Fatty acid degradation
Ascorbate and aldarate metabolism
beta-Alanine metabolism
Glycolysis / Gluconeogenesis
Steroid biosynthesis
Mitophagy - yeast
Tyrosine metabolism
Glutathione metabolism
Cyanoamino acid metabolism
Sulfur relay system
Phenylalanine metabolism
Thiamine metabolism
ABC transporters
Tryptophan metabolism
Histidine metabolism
Citrate cycle (TCA cycle)
Valine, leucine and isoleucine degradation
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Rich factor
Pvalue
0.076
0.038
0
Number
3
4
14

B

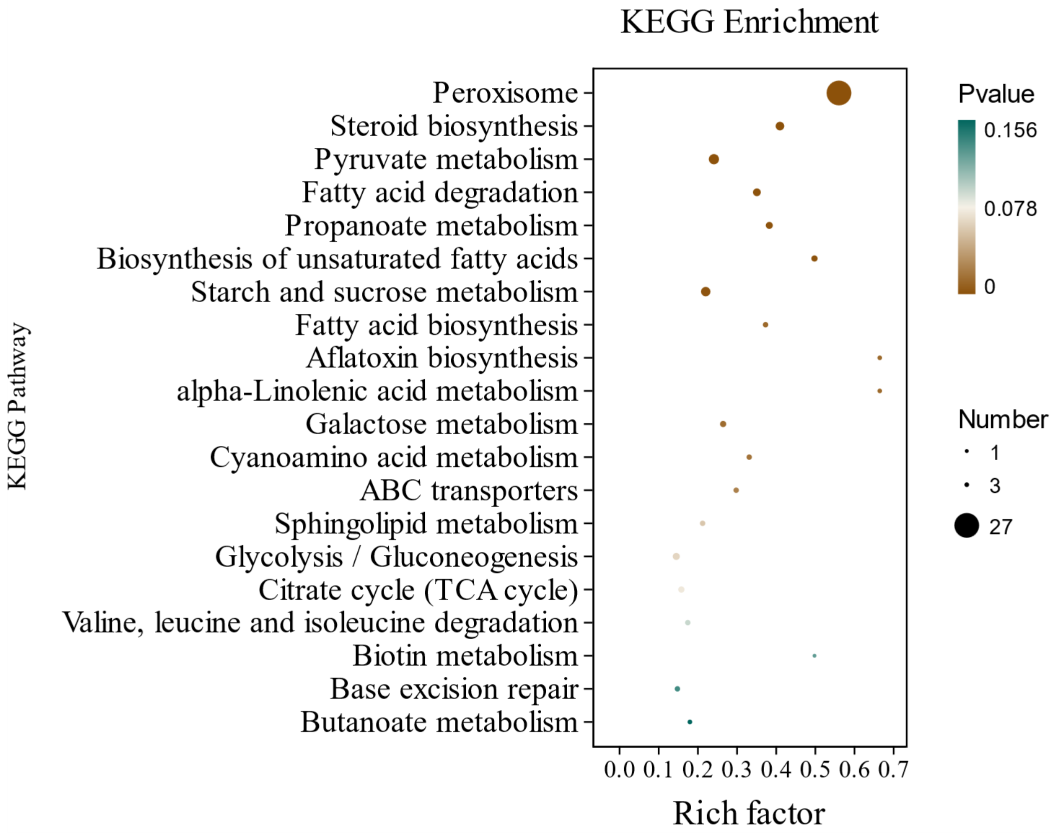

KEGG Enrichment
KEGG Pathway
Peroxisome
Steroid biosynthesis
Pyruvate metabolism
Fatty acid degradation
Propanoate metabolism
Biosynthesis of unsaturated fatty acids
Starch and sucrose metabolism
Fatty acid biosynthesis
Aflatoxin biosynthesis
alpha-Linolenic acid metabolism
Galactose metabolism
Cyanoamino acid metabolism
ABC transporters
Sphingolipid metabolism
Glycolysis / Gluconeogenesis
Citrate cycle (TCA cycle)
Valine, leucine and isoleucine degradation
Biotin metabolism
Base excision repair
Butanoate metabolism
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Rich factor
Pvalue
0.156
0.078
0
Number
1
3
27

Table S1

Transcriptome data quality statistics.

sample	Raw_Read_Number	Raw_Bases	Raw_Q20_Rate	Raw_Q30_Rate	Useful_read%	Useful_bases%
A35_1	48042684	7254445284	98.02	94.46	98.50	98.37
A35_2	49961406	7544172306	97.85	94.05	98.37	98.22
A35_3	47446656	7164445056	97.94	94.25	98.45	98.31
B35_1	52591860	7941370860	98.16	94.79	98.60	98.46
B35_2	48368232	7303603032	97.87	94.11	98.35	98.21
B35_3	49750124	7512268724	98.13	94.75	98.54	98.39
A94_1	49905082	7535667382	97.82	93.95	98.38	98.27
A94_2	45213324	6827211924	97.94	94.25	98.49	98.35
A94_3	45822160	6919146160	97.91	94.21	98.42	98.30
B94_1	48979232	7395864032	97.91	94.17	98.48	98.34
B94_2	51102186	7716430086	97.99	94.37	98.52	98.38
B94_3	46943398	7088453098	98.15	94.76	98.64	98.49

Table S2

Reference genome comparison results

sample	Clean_Reads	Total_Mapped	Multiple_Mapped	Uniquely_Mapped
A35_1	47322812	98.25%	2.45%	97.55%
A35_2	49146832	98.19%	2.48%	97.52%
A35_3	46709620	98.25%	2.44%	97.56%
B35_1	51855188	98.17%	2.38%	97.62%
B35_2	47571500	98.06%	2.32%	97.68%
B35_3	49022214	98.14%	2.33%	97.67%
A94_1	49099072	98.11%	2.37%	97.63%
A94_2	44528948	98.06%	2.38%	97.62%
A94_3	45098386	98.09%	2.41%	97.59%
B94_1	48233764	98.12%	2.22%	97.78%
B94_2	50347726	98.03%	2.22%	97.78%
B94_3	46303488	98.14%	2.19%	97.81%

Table S3

Enrichment analysis of differential genes in pyruvate metabolism.

Characteristic	Abridge	Name	35 h	94 h
Pyruvate metabolism	11dD	L-lactate dehydrogenase	-2.41	+2.44
	acs	acetyl-CoA synthetase	-1.52	/
	LDHD	D-lactate dehydrogenase	-1.25	+1.72
	aldh	aldehyde dehydrogenase	/	-1.47
	adhp	alcohol dehydrogenase		-1.6
Amino acid degradation	ALDH	aldehyde dehydrogenase	-3.62	/
	fadi	acetyl-CoA acyltransferase	-1.74	/
	EGT2	hercynylcysteine S-oxide lyase	-1.68	/
Glutathione metabolism	PepN	aminopeptidase N	-1.45	/
	ggt	glutathione hydrolase	-1.04	/
	gst	glutathione S-transferase	-1.61	+2.34
	Trys	trypanothione synthetase/amidase	-2.23	/
	TryR	trypanothione-disulfide reductase	-1.64	/
	RRM1	ribonucleoside-diphosphate reductase subunit M1	-1.3	/
Peroxisome biogenesis	PTS2	peroxisomal long-chain fatty acid import protein	-2.14	+2.79
	PEX5	peroxin-5	-1.88	+2.76
	PTS1	carnitine O-acetyltransferase	-1.91	+1.66
	PEX13	peroxin-13	-1.4	+1.75

	PEX12	peroxin-12	/	+1.94
	PEX10	peroxin-10	-2.8	/
	PEX2	peroxin-2	/	+1.76
	PEX6	peroxin-6	/	+2.03
	PEX1	peroxin-1	/	+1.15
	PEX16	peroxin-16	/	+2.83
	PEX3	peroxin-3	/	+1.94
	PEX19	peroxin-19	-1.67	+2.03
	PXMP4	peroxisomal membrane protein 4	-1.65	+2.48
	PMP34	peroxisomal adenine nucleotide transporter	-2.22	+2.96
Terpenoid backbone biosynthesis	HMGCS	hydroxymethylglutaryl-CoA synthase	/	+1.98
	FDPS	farnesyl diphosphate synthase	-1.81	/
	heps	heptaprenyl diphosphate synthase	-1.29	/
	GGPS1	geranylgeranyl diphosphate synthase	-1.64	+1.71
	ERG6	sterol 24-C-methyltransferase	-1.41	
	ERG7	lanosterol synthase	-2.21	-2.2
	ERG24	Delta14-sterol reductase	/	-2.13
	ERG25	methylsterol monooxygenase	/	1.8
	ERG3	Delta7-sterol 5-desaturase	/	1.73

	ERG7	lanosterol synthase	-2.21	-2.2
	ERG2	C-8 sterol isomerase	/	-1.16
	ERG4	Delta24(24(1))-sterol reductase	+2.06	-1.24
Mitophagy-yeast	ATG32	autophagy-related protein 32	+2.13	/
	DNM1	dynamin 1-like protein	+1.99	/
	F1S1	mitochondrial fission 1 protein	+1.8	/
	MDM34	mitochondrial distribution and morphology protein 34	+2.78	/
	MDM10	mitochondrial distribution and morphology protein 10	+2.23	/
Fatty acid biosynthesis	ACSL	long-chain acyl-CoA synthetase	/	+2.61
	Fas1	fatty acid synthase subunit beta	/	-1.86
	ACACA	acetyl-CoA carboxylase	/	-1.64
Fatty acid degradation	ACSL	long-chain acyl-CoA synthetase	/	+2.61
	acd	acyl-CoA dehydrogenase	/	+2.98
	ACOX1	acyl-CoA oxidase	/	+3.16
	fadA	acetyl-CoA acyltransferase	-2.08	+2.79

Table S4

Name	Sequences
18S-F	TTGAACTTCAGGCCTGGTCG
18S-R	AGGCCTGCTTTGAACACTCT
PTS2-F	GATGGCTGTGATCTCTGGAGG
PTS2-R	CTTGGAGGGATGTGGTCAAAGA
PEX5-F	TTCTGCTCAACGAGGAAAAGGC
PEX5-R	CATCGTCTGAGGATCGAACGC
PEX19-F	ATGATTTGGATGACTTGATCCCTTCG
PEX19-R	CTTCATCTCCATCCTCCCCGG
PMP34-F	TTAGCAGTTCGATTGCCGTTACAT
PMP34-R	AGAAGAAGTAGGCATACTGCATAGAG
gst-F	AACACTATTGCCGAGTCCGG
gst-R	GCATCAAACTACCCTCGGCA
TRYS-F	actggtgcgtaagggactgt
TRYS-R	atcggtggaggggtagacaag
ATG32-F	tgaacttaatcaagacattaggcatctgac
ATG32-R	cagttccgcatttgcaaatatgtct
MDM10-F	CGGTCTTTATACAAGCAGGCTCTC
MDM10-R	AAAGAGAAGGCTGGATTTGACGG
MDM34-F	GTTCTCTCATTCGTTGTTCAGATCAGC
MDM34-R	ATCATCAGGTGGAGGTTCAGGAG
ACSL-F	CGACAAGCTCGAATGGATCAGC
ACSL-R	AGATACCGACACCCCATCCAC
ERG7-F	CATAAGCTCTCCAACGCTATCTCTCC
ERG7-R	CACCATCCGATAGTTCCTTTCTAAGGC
GGPS1-F	GGACGGGAAGAAGAAGCTCTACA
GGPS1-R	GCCAAAGACGCGGTAATGAG
ERG4-F	ACATTTTCGATACCGCAAACTCCC
ERG4-R	TAAGAAGCTTGCTACCATGCTGAGT
acd-F	TGGCTGATTTGACGGGAGCAAA
acd-R	ATCGAACGAGGAGACATCCTCA
fadA-F	GAGGCTTCGACCGATTCTACTACC
fadA-R	CGAGGAAAGGAGCGTTGGAC
11dh-F	CTCTGCTCTCACATTGGCAGC
11dh-R	GATCCATAGGCTGTCTTTGGTATTGT