

**Effects of Methanol on Biomass as Well as Fatty Acid and Carotenoid Biosynthesis in  
*Schizochytrium limacinum* B4D1**

Huanmin Du<sup>1,2,3#</sup>, Xiaoping Liao<sup>2#</sup>, Zhengquan Gao<sup>1#</sup>, Yang Li<sup>2#</sup>, Yu Lei<sup>2</sup>, Wuxi Chen<sup>2</sup>, Limei Chen<sup>2</sup>, Xiang Fan<sup>2</sup>, Ke Zhang<sup>2</sup>, Shulin Chen<sup>2</sup>, Yanhe Ma<sup>2</sup>, Chunxiao Meng<sup>1\*</sup>, Demao Li<sup>2\*</sup>

1 School of Life Sciences, Shandong University of Technology, Zibo, 255049, China.

2 Tianjin Key Laboratory for Industrial Biological Systems and Bioprocessing Engineering, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308, China.

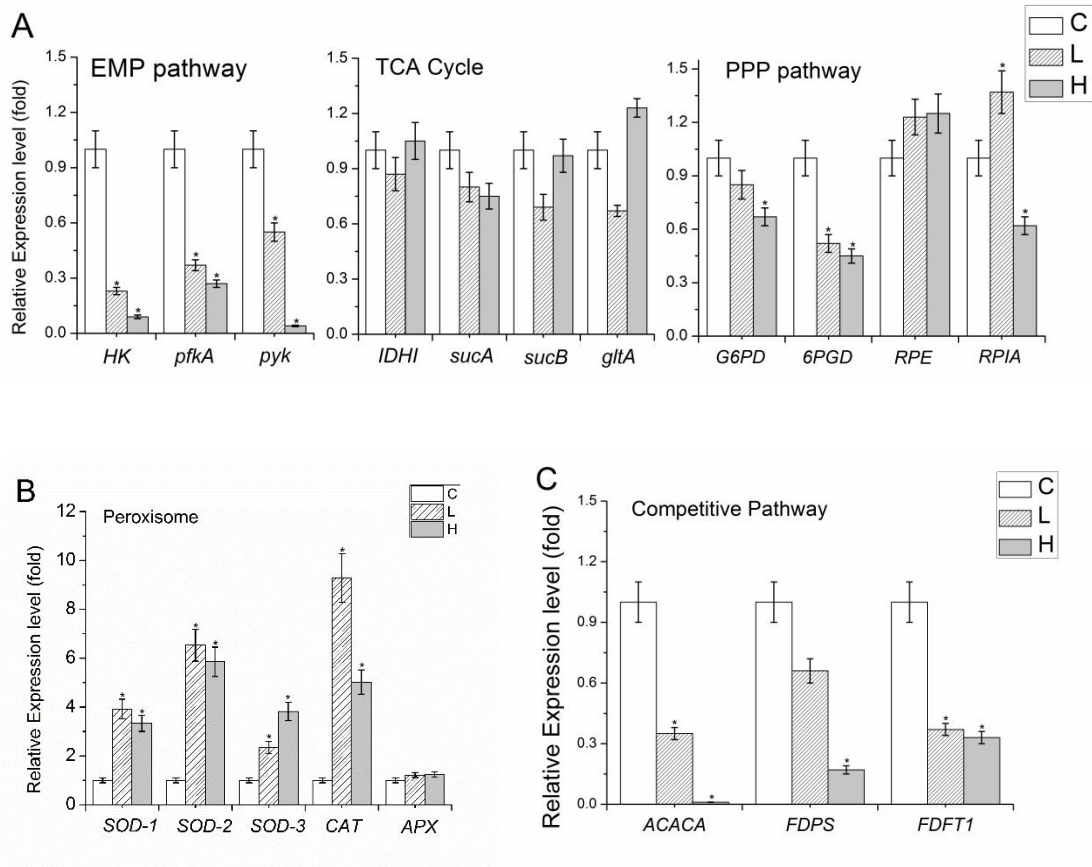
3 University of Chinese Academy of Sciences, Beijing 100049, China.

# Huanmin Du, Xiaoping Liao, Zhengquan Gao and Yang Li contributed equally to this work.

\* Corresponding authors:

Chunxiao Meng, mengchunxiao@126.com

Demao Li, li\_dm@tib.cas.cn



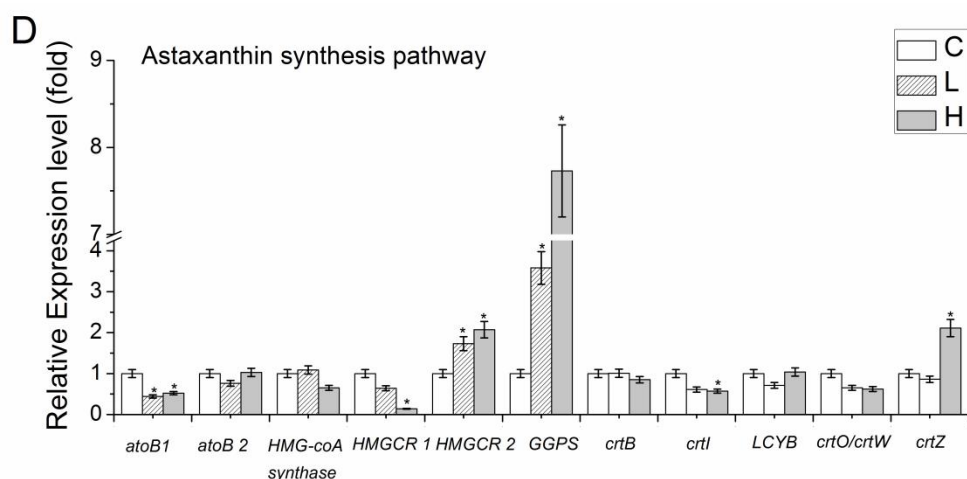


FIG S1 Quantitative Real-time PCR results for genes from the *S. limacinum* B4D1. A: key genes in central carbon metabolism pathways; B: key genes in antioxidant defense system; C: key genes in fatty acid synthesis pathway and squalene/sterols synthesis pathway; C: key genes in carotenoids biosynthesis pathway. Shown are mean values and SEs from three separately grown cultures. The “\*” indicate statistically significant differences compared to controls.

TABLE S1 The genes list mentioned in this paper

Gene name	Function
<i>HRAS</i>	GTPase HRas
<i>KRAS</i>	GTPase KRas
<i>NRAS</i>	GTPase NRas
<i>MRAS</i>	GTPase MRas
<i>RRAS</i>	GTPase RRas
<i>PLD</i>	phospholipase D1/2
<i>AMPK</i>	5'-AMP-activated protein kinase
<i>SREBP1</i>	sterol regulatory element-binding transcription factor 1
<i>pct</i>	propionate CoA-transferase
<i>DLAT</i>	pyruvate dehydrogenase E2 component
<i>pta</i>	phosphate acetyltransferase
<i>gapA-1</i>	glyceraldehyde 3-phosphate dehydrogenase
<i>gapA-2</i>	
<i>gapA-3</i>	
<i>HK</i>	hexokinase
<i>pfkA</i>	6-phosphofructokinase
<i>Pyk-1</i>	pyruvate kinase
<i>Pyk-2</i>	
<i>Pyk-3</i>	
<i>Pgk-1</i>	phosphoglycerate kinase
<i>Pgk-2</i>	
<i>ALDO</i>	fructose-bisphosphate aldolase
<i>eno-1</i>	enolase

<i>eno-2</i>	
<i>tpiA-1</i>	triosephosphate isomerase (TIM)
<i>tpiA-2</i>	
<i>Pgi</i>	glucose-6-phosphate isomerase
<i>ADPGK</i>	ADP-dependent glucokinase
<i>gpmI</i>	2,3-bisphosphoglycerate-independent phosphoglycerate mase
<i>gpmB</i>	phosphoglycerate mutase
<i>ME-1</i>	malic enzyme
<i>ME-2</i>	
<i>IDH3</i>	isocitrate dehydrogenase
<i>IDH1</i>	isocitrate dehydrogenase (NAD <sup>+</sup> )
<i>sucA-1</i>	2-oxoglutarate dehydrogenase E1 component
<i>sucA-2</i>	
<i>sucB-1</i>	2-oxoglutarate dehydrogenase E2 component (dihydrolipo amide
<i>sucB-2</i>	succinyltransferase)
<i>sucB-3</i>	
<i>korA</i>	2-oxoglutarate ferredoxin oxidoreductase subunit alpha
<i>SDH1</i>	succinate dehydrogenase (ubiquinone) flavoprotein subunit
<i>SDH3</i>	succinate dehydrogenase (ubiquinone) cytochrome b560 subunit
<i>frdA</i>	fumarate reductase flavoprotein subunit
<i>pdhD</i>	dihydrolipoamide dehydrogenase
<i>gltA-1</i>	citrate synthase
<i>gltA-2</i>	
<i>fumC-1</i>	fumarate hydratase
<i>fumC-2</i>	
<i>acnB</i>	aconitate hydratase 2 / 2-methylisocitrate dehydratase
<i>LSC2-1</i>	succinyl-CoA synthetase beta subunit
<i>LSC2-2</i>	
<i>G6PD</i>	Glucose-6-phosphate 1-dehydrogenase
<i>6PGD</i>	6-phosphogluconate dehydrogenase
<i>RPE</i>	Ribulose-phosphate 3-epimerase
<i>RPIA</i>	Ribose 5-phosphate epimerase
<i>SOD-1</i>	Superoxide dismutase
<i>SOD-2</i>	
<i>SOD-3</i>	
<i>SOD-4</i>	
<i>SOD-5</i>	
<i>SOD-6</i>	
<i>CAT</i>	Catalase
<i>APX</i>	L-ascorbate peroxidase
<i>GSH-Px-1</i>	Glutathione peroxidase
<i>GSH-Px-2</i>	
<i>GSH-Px-3</i>	

<i>ACACA</i>	acetyl-CoA carboxylase / biotin carboxylase
<i>accC</i>	acetyl-CoA carboxylase, biotin carboxylase subunit
<i>FASN</i>	fatty acid synthase, animal type
<i>accD</i>	acetyl-CoA carboxylase carboxyl transferase subunit
<i>pks-1</i>	polyketide synthase
<i>pks-2</i>	
<i>FDPS</i>	farnesyl diphosphate synthase
<i>FDFT1</i>	farnesyl-diphosphate farnesyltransferase
<i>AtoB-1</i>	acetyl-CoA C-acetyltransferase
<i>AtoB-2</i>	
<i>AtoB-3</i>	
<i>AtoB-4</i>	
<i>AtoB-5</i>	
<i>HMG-CoA synthase</i>	hydroxymethylglutaryl-CoA synthase
<i>HMGCR-1</i>	hydroxymethylglutaryl-CoA reductas
<i>HMGCR-2</i>	
<i>GGPS</i>	geranylgeranyl diphosphate synthase
<i>crtB</i>	15-cis-phytoene synthase
<i>crtI</i>	phytoene desaturase
<i>LCYB</i>	lycopene beta-cyclase
<i>crtO/crtW</i>	beta-carotene ketolase
<i>crtZ</i>	beta-carotene 3-hydroxylase

TABLE S2 Expression of key genes related to central carbon metabolism.

Pathway	Gene name	FPKM			$\log_2(L/C)$	$\log_2(H/C)$	$\log_2(H/L)$
		C	L	H			
EMP	<i>gapA-1</i>	5364.28	2208.31	724.52	-1.28	-2.89	-1.61
	<i>gapA-2</i>	3432.6	1763.39	595.88	-0.96	-2.53	-1.57
	<i>gapA-3</i>	198.13	195.57	39.07	-0.02	-2.34	-2.32
	<i>HK</i>	466.13	146.33	64.59	-1.67	-2.85	-1.18
	<i>pfkA</i>	510.83	305.36	155.97	-0.74	-1.71	-0.97
	<i>Pyk-1</i>	1727.62	852.57	132.91	-1.02	-3.70	-2.68
	<i>Pyk-2</i>	213.76	147.27	113.6	-0.54	-0.91	-0.37

	<i>Pyk-3</i>	2.03	3.58	13.89	0.82	2.77	1.96
	<i>Pgk-1</i>	949.57	732.28	573.64	-0.37	-0.73	-0.35
	<i>Pgk-2</i>	616.58	444.15	264.97	-0.47	-1.22	-0.75
	<i>ALDO</i>	8790.6	2690.33	743.3	-1.71	-3.56	-1.86
	<i>eno-1</i>	2784.57	1388.27	555.09	-1.00	-2.33	-1.32
	<i>eno-2</i>	54.52	45.74	107.5	-0.25	0.98	1.23
	<i>tpiA-1</i>	2192.19	688.27	284.28	-1.67	-2.95	-1.28
	<i>tpiA-2</i>	1425.02	721.24	220.65	-0.98	-2.69	-1.71
	<i>Pgi</i>	399.59	274.67	51.96	-0.54	-2.94	-2.40
	<i>ADPGK</i>	12.11	17.58	21.05	0.54	0.80	0.26
	<i>gpmI</i>	707.81	453.16	317.82	-0.64	-1.16	-0.51
	<i>gpmB</i>	43.37	75.22	198.53	0.79	2.19	1.40
TCA	<i>ME-1</i>	1348.18	1312.64	692.66	-0.04	-0.96	-0.92
	<i>ME-2</i>	722.9	633.33	269.43	-0.19	-1.42	-1.23
	<i>IDH3</i>	399.6	351.85	846.6	-0.18	1.08	1.27
	<i>IDH1</i>	17.36	19.13	26.46	0.14	0.61	0.47
	<i>sucA-1</i>	347.428	282.597	302.563	-0.30	-0.20	0.10
	<i>sucA-2</i>	11.4292	10.3657	41.8307	-0.14	1.87	2.01
	<i>sucB-1</i>	311.746	249.941	318.765	-0.32	0.03	0.35
	<i>sucB-2</i>	296.327	116.768	222.555	-1.34	-0.41	0.93
	<i>sucB-3</i>	65.0034	66.3826	65.1986	0.03	0.00	-0.03
	<i>korA</i>	18.15	8.31	5.56	-1.13	-1.71	-0.58
	<i>SDH1</i>	464.96	318.59	260.16	-0.55	-0.84	-0.29
	<i>SDH3</i>	1036.45	888.34	426.31	-0.22	-1.28	-1.06
	<i>frdA</i>	10.14	18.11	24.41	0.84	1.27	0.43
	<i>pdhD</i>	901.51	470.66	382.27	-0.94	-1.24	-0.30
	<i>gltA-1</i>	995.39	811.542	1133.61	-0.29	0.19	0.48
	<i>gltA-2</i>	29.0187	31.0607	39.8868	0.10	0.46	0.36
	<i>fumC-1</i>	178.37	170.15	289.56	-0.07	0.70	0.77
	<i>fumC-2</i>	6.77	9.71	11.61	0.52	0.78	0.26
	<i>acnB</i>	1246.08	1178.33	522.3	-0.08	-1.25	-1.17
	<i>LSC2-1</i>	349.19	471.54	434.78	0.43	0.32	-0.12
<i>LSC2-2</i>	119.06	66.32	272.7	-0.84	1.20	2.04	
PPP	<i>G6PD</i>	297.151	255.485	198.068	-0.36	-0.22	-0.58
	<i>6PGD</i>	993.297	466.975	385.978	-0.27	-1.09	-1.36
	<i>RPE</i>	94.9946	109.717	115.308	0.07	0.20	0.28
	<i>RPIA</i>	68.0836	105.278	39.6767	-1.40	0.63	-0.79

TABLE S3 Expression of key genes related to antioxidant defense system

Gene name	FPKM			$\log_2(L/C)$	$\log_2(H/C)$	$\log_2(H/L)$
	C	L	H			
<i>SOD-1</i>	561.68	2126.22	1823.49	1.92	1.7	-0.22
<i>SOD-2</i>	96.9404	645.232	561.171	2.74	2.53	-0.2

<i>SOD-3</i>	149.988	376.907	561.995	1.33	1.91	0.58
<i>SOD-4</i>	200.164	213.846	243.229	0.10	0.28	0.19
<i>SOD-5</i>	50.2632	67.5072	46.3508	0.42	-0.12	-0.54
<i>SOD-6</i>	49.1659	36.6054	46.9436	-0.43	-0.07	0.36
<i>CAT</i>	54.4367	509.177	269.323	3.22	2.31	-0.92
<i>APX</i>	179.364	226.395	216.565	0.33	0.28	-0.06
<i>GSH-Px-1</i>	99.6278	115.014	93.4697	0.2	-0.09	-0.3
<i>GSH-Px-2</i>	194.68	143.276	60.8679	-0.45	-1.69	-1.25
<i>GSH-Px-3</i>	510.021	336.96	203.983	-0.6	-1.32	-0.71

TABLE S4 Expression of key genes related to two competitive pathways

Gene name	FPKM			$\log_2(L/C)$	$\log_2(H/C)$	$\log_2(H/L)$
	C	L	H			
<i>ACACA</i>	1032.18	489.24	10.14	-1.08	-6.67	-5.59
<i>accC</i>	39.81	13.94	21.86	-1.51	-0.87	0.65
<i>FASN</i>	24.13	34.49	0	0.52	-	-
<i>accD</i>	11.19	3.02	1.09	-1.89	-3.36	-1.47
<i>pks-1</i>	895.78	532.16	6.4	-0.75	-7.13	-6.38
<i>pks-2</i>	561.03	278.54	21.08	-1.01	-4.73	-3.72
<i>FDPS</i>	282.29	182.21	56.31	-0.63	-2.33	-1.69
<i>FDFT1</i>	100.37	88.73	29.42	-0.18	-1.77	-1.59

TABLE S5 Expression of key genes related to carotenoid pathway analysis

Gene name	FPKM			$\log_2(L/C)$	$\log_2(H/C)$	$\log_2(H/L)$
	C	L	H			
<i>AtoB-1</i>	158.86	176.04	144.77	0.15	-0.13	-0.28
<i>AtoB-2</i>	134.06	136.80	81.04	0.03	-0.73	-0.76
<i>AtoB-3</i>	25.22	31.64	24.01	0.33	-0.07	-0.40
<i>AtoB-4</i>	5.24	8.00	37.95	0.61	2.85	2.25
<i>AtoB-5</i>	2.19	0.49	1.76	-2.17	-0.32	1.85
<i>HMG-CoA synthase</i>	127.80	197.97	92.27	0.63	-0.47	-1.10
<i>HMGCR-1</i>	511.66	219.34	49.56	-1.22	-3.37	-2.15
<i>HMGCR-2</i>	57.40	96.16	111.41	0.74	0.96	0.21
<i>GGPS</i>	37.82	98.54	241.32	1.38	2.67	1.29
<i>crtB</i>	43.26	43.83	37.57	0.02	-0.20	-0.22

<i>crtI</i>	16.91	10.84	7.26	-0.64	-1.22	-0.58
<i>LCYB</i>	128.34	129.03	153.08	-0.22	-1.56	-1.34
<i>crtO/crtW</i>	27.87	27.52	18.70	-0.02	-0.58	-0.56
<i>crtZ</i>	8.93	13.11	21.72	0.55	1.28	0.73