

Selecting a preculture strategy for improving biomass and astaxanthin productivity of *Chromochloris*
zofingiensis

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Fig. S3. Transcriptional regulation of “photosynthesis-antenna protein” KEGG pathway. The red and blue boxes indicate up-regulation and down-regulation respectively.

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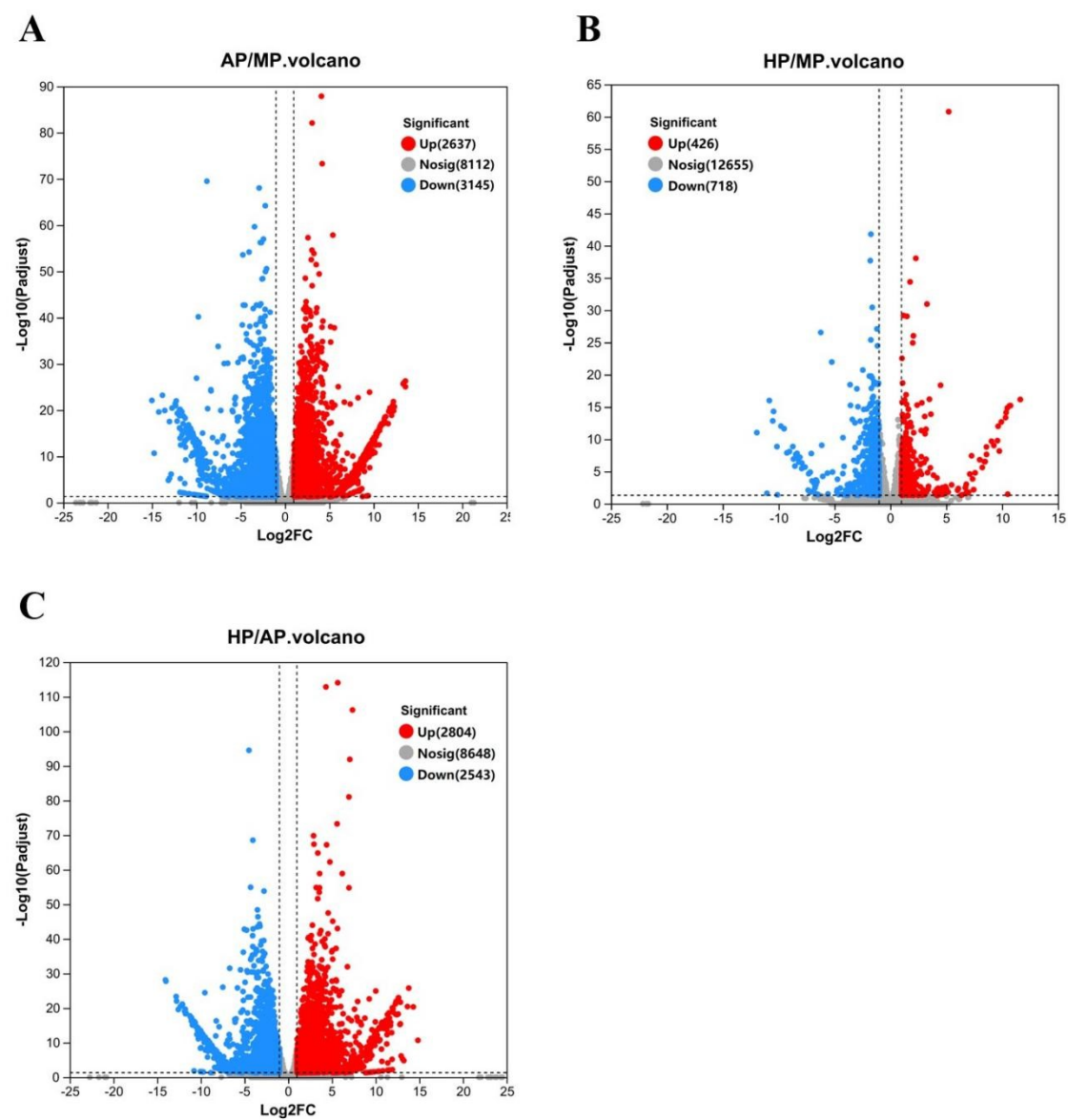


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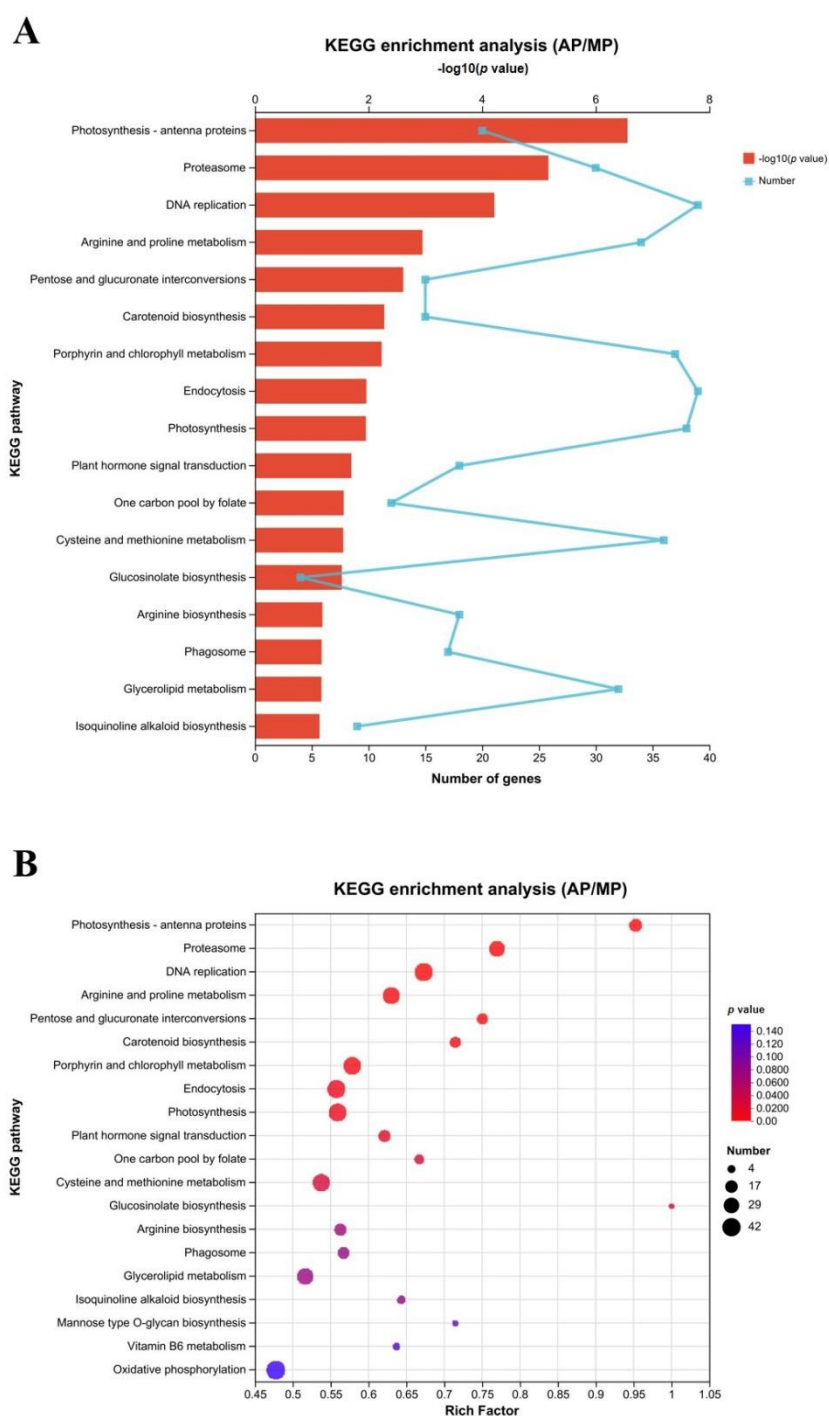


Fig. S3. Transcriptional regulation of of AP/MP KEGG pathway of “photosynthesis-antenna protein”. The red and blue boxes indicate up-regulation and down-regulation respectively.

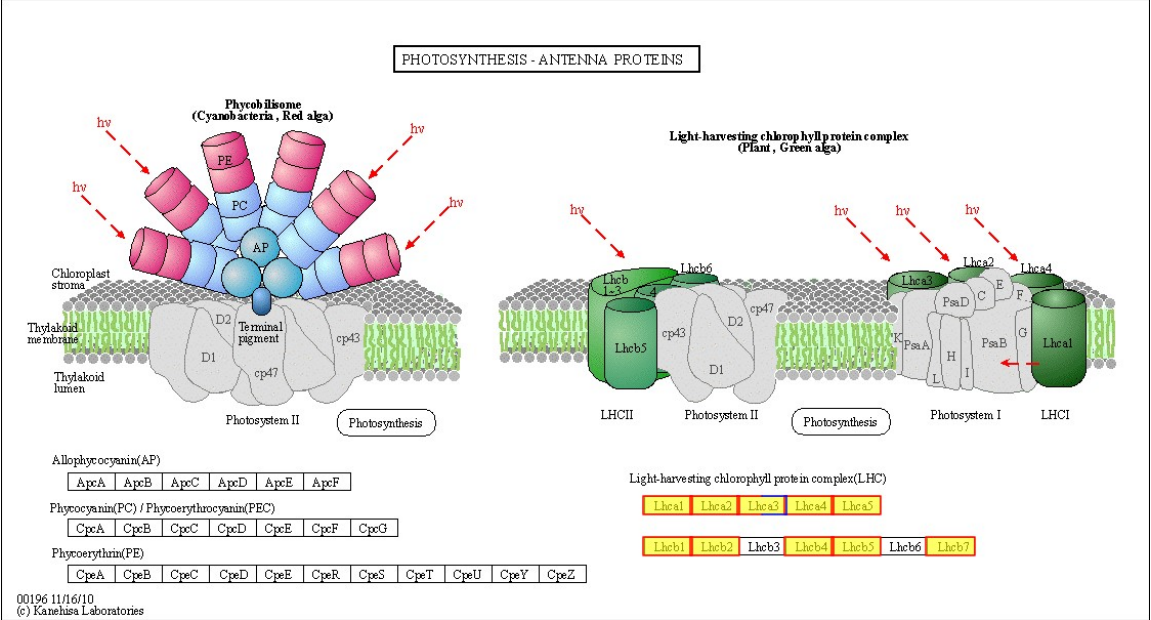


Table S1 Expression patterns of genes involved in TAG, astaxanthin biosynthesis and central carbon mechanism

Gene ID	Annotation	Abbreviation	log 2 FC		
			AP/MP	HP/AP	HP/MP
FA biosynthesis					
Cz02g12030	Carboxyltransferase subunit alpha (ACCase complex)	<i>α-CT</i>	-0.45	0.73	0.29
Cz02g17060	Carboxyltransferase subunit beta (ACCase complex)	<i>β-CT</i>	-0.05	0.55	0.51
Cz03g28270	Biotin carboxyl carrier protein (ACCase complex)	<i>BCCP1</i>	-0.83	0.80	-0.02
Cz06g20040	Biotin carboxyl carrier protein (ACCase complex)	<i>BCCP2</i>	0.74	0.09	0.83
Cz13g10110	Biotin carboxylase (ACCase complex)	<i>BC</i>	-1.53	1.38	-0.13
Cz19g10190	Acetyl-CoA carboxylase	<i>ACCase</i>	-0.59	0.79	0.22
Cz09g30220	Acyl carrier protein	<i>ACP1</i>	-0.89	0.93	0.05
Cz07g17120	Acyl carrier protein	<i>ACP2</i>	-1.11	0.98	-0.11
Cz13g05150	Malonyl-CoA:Acyl carrier protein transacylase	<i>MCT1</i>	-2.29	2.10	-0.17
Cz04g37050	Malonyl-CoA:Acyl carrier protein transacylase	<i>MCT2</i>	0.99	-2.29	-1.27
UNPLg00257	3-Oxoacyl-[acyl-carrier-protein] synthase, II (C16:0 to C18:0)	<i>KAS II</i>	-1.54	1.25	-0.27
Cz02g14160	3-Oxoacyl-[acyl-carrier-protein] synthase, I (C4:0 to up to C16:0)	<i>KAS I</i>	-3.17	2.78	-0.37
Cz18g03070	3-Oxoacyl-[acyl-carrier-protein] synthase, III (C2:0 to C4:0)	<i>KAS III</i>	-1.00	1.05	0.07
Cz06g14030	3-Oxoacyl-[acyl-carrier-protein] synthase, II	<i>KAS II</i>	-0.66	0.69	0.04
Cz01g34370	3-Oxoacyl-[acyl-carrier protein] reductase	<i>KAR1</i>	-2.48	2.53	0.06
Cz16g00050	3-Oxoacyl-[acyl-carrier protein] reductase	<i>KAR2</i>	-0.64	0.56	-0.06
Cz10g18200	3-Oxoacyl-[acyl-carrier protein] reductase	<i>KAR3</i>	1.55	-1.10	0.47
Cz03g39140	3-Oxoacyl-[acyl-carrier protein] reductase?	<i>KAR4</i>	1.48	-2.06	-0.57
Cz15g00050	3-Oxoacyl-[acyl-carrier protein] reductase	<i>KAR5</i>	-0.43	-0.09	-0.51
Cz04g17270	3-Oxoacyl-[acyl-carrier protein] reductase	<i>KAR6</i>	1.82	-1.20	-0.60
Cz11g27250	3-Oxoacyl-[acyl-carrier protein] reductase?	<i>KAR7</i>	0.56	-1.33	-0.75
Cz01g09160	3-Hydroxyacyl-[acyl-carrier-protein] dehydratase	<i>HAD</i>	-2.87	1.79	-1.06
Cz11g20040	Enoyl-[acyl-carrier protein] reductase	<i>ENR</i>	-2.69	1.54	-1.14
FA desaturation					
Cz04g09090	Acyl-[acyl-carrier-protein] desaturase	<i>SAD1</i>	-4.16	3.35	-0.79
Cz13g17200	Acyl-[acyl-carrier-protein] desaturase	<i>SAD2</i>	0.47	-0.92	-0.44

TAG synthesis

Cz11g03260	Glycerol-3-phosphate acyltransferase	<i>GPAT1</i>	8.96	-9.90	-0.91
Cz09g31330	Glycerol-3-phosphate acyltransferase	<i>GPAT2</i>	-0.97	0.93	-0.02
Cz16g02090	1-Acyl- <i>sn</i> -glycerol-3-phosphate acyltransferase	<i>LPAAT1</i>	10.00	-10.00	0.02
Cz10g20070	1-Acyl- <i>sn</i> -glycerol-3-phosphate acyltransferase	<i>LPAAT2</i>	0.17	-0.27	-0.08
Cz05g23060	Phosphatidate phosphatase, Lipin	<i>PAP1</i>	0.70	-0.19	0.53
Cz10g16040	Phosphatidate phosphatase	<i>PAP2</i>	5.24	-4.97	0.29
Cz16g11240	Phosphatidate phosphatase	<i>PAP3</i>	-1.78	3.69	1.91
Cz10g01120	2-Acylglycerol <i>O</i> -acyltransferase	<i>MGAT</i>	7.13	-7.38	-0.22
Cz06g05010	Diacylglycerol <i>O</i> -acyltransferase, type I	<i>DGAT1A</i>	-2.15	2.05	-0.08
Cz09g08290	Diacylglycerol <i>O</i> -acyltransferase, type I	<i>DGAT1B</i>	1.78	-1.53	0.27
Cz06g35060	Diacylglycerol <i>O</i> -acyltransferase, type II	<i>DGTT1</i>	-0.88	1.76	0.91
Cz06g22030	Diacylglycerol <i>O</i> -acyltransferase, type II	<i>DGTT2</i>	-0.46	-0.35	-0.78
Cz09g23010	Diacylglycerol <i>O</i> -acyltransferase, type II	<i>DGTT3</i>	-0.56	3.49	2.95
Cz11g24150	Diacylglycerol <i>O</i> -acyltransferase, type II	<i>DGTT4</i>	2.73	-3.24	-0.49
Cz09g27290	Diacylglycerol <i>O</i> -acyltransferase, type II	<i>DGTT5</i>	-3.16	2.56	-0.59
Cz15g22140	Diacylglycerol <i>O</i> -acyltransferase, type II	<i>DGTT6</i>	-12.26	12.35	0.00
Cz11g21100	Diacylglycerol <i>O</i> -acyltransferase, type II	<i>DGTT7</i>	-1.89	0.52	-1.35
Cz08g14220	Phospholipid:diacylglycerol acyltransferase	<i>DGTT8</i>	-0.01	0.21	0.21

Carotenoids synthesis

Cz02g35280	1-Deoxy-D-xylulose 5-phosphate synthase (DOXP synthase)	<i>DXS</i>	1.25	-1.02	0.24
Cz07g19130	1-Deoxy-D-xylulose 5-phosphate reductoisomerase	<i>DXR</i>	0.60	-1.01	-0.40
Cz12g10090	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	<i>CMS</i>	9.12	-8.48	0.67
Cz11g24270	4-Diphosphocytidyl-2-C-methyl-D-erythritol kinase	<i>CMK</i>	-0.02	-0.26	-0.27
Cz02g10180	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	<i>MCS</i>	5.23	-5.92	-0.68
Cz13g08020	4-Hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	<i>HDS</i>	0.36	-0.60	-0.23
Cz05g23010	4-Hydroxy-3-methylbut-2-enyl diphosphate reductase	<i>HDR</i>	0.57	-0.88	-0.30
Cz04g25080	Acetoacetyl-CoA thiolase	<i>AACT</i>	-0.70	0.28	-0.40
Cz19g02060	Hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase)	<i>HCS</i>	1.12	-1.67	-0.54
Cz03g02150	Isopentenyl-diphosphate Delta-isomerase	<i>IPPI</i>	0.37	0.13	0.52
Cz02g31110	Geranyl diphosphate synthase	<i>GPPS</i>	0.32	-0.22	0.12
Cz01g03190	Farnesyl diphosphate synthase	<i>FPPS</i>	-0.82	0.63	-0.18
Cz02g19200	Geranylgeranyl diphosphate synthase	<i>GGPPS</i>	1.76	-1.81	-0.03
Cz05g32220	Phytoene synthase (chloroplastic)	<i>PSY</i>	5.62	-5.24	0.40

Cz02g32280	Phytoene desaturase	<i>PDS</i>	3.47	-2.64	0.86
Cz10g17010	Zeta-carotene desaturase (chloroplastic)	<i>ZDS</i>	2.22	-1.68	0.55
Cz10g17130	Zeta-carotene isomerase	<i>ZISO</i>	2.38	-1.74	0.65
Cz16g01210	Carotenoid isomerase	<i>CRTISO1</i>	3.33	-3.53	-0.17
Cz12g03260	Carotenoid isomerase	<i>CRTISO2</i>	-1.19	1.19	0.02
Cz14g22040	Carotenoid isomerase	<i>CRTISO3</i>	1.61	-1.33	0.29
Cz09g18310	Lycopene epsilon cyclase	<i>LCYe</i>	1.25	-1.86	-0.59
Cz12g10170	Lycopene beta cyclase	<i>LCYb</i>	0.70	-0.18	0.54
Cz13g16110	Cytochrome P450 beta hydroxylase, CYP97A5(Cr)	<i>CYP97A</i>	4.03	-3.57	0.48
Cz09g07100	Cytochrome P450 epsilon hydroxylase, CYP97C3	<i>CYP97C</i>	2.44	-2.76	-0.30
Cz09g14130	Cytochrome P450 CYP97B, beta or seta-rings (At)	<i>CYP97A</i>	0.74	-0.64	0.12
Cz12g16080	Beta-carotene hydroxylase	<i>CHYb</i>	0.45	-0.18	0.28
Cz13g13100	Beta-carotene ketolase/oxygenase	<i>BKT1</i>	1.23	-0.74	0.50
Cz04g11250	Beta-carotene ketolase/oxygenase	<i>BKT2</i>	0.95	-0.46	0.50
Cz07g30060	Zeaxanthin epoxidase	<i>ZEP</i>	2.18	-1.83	0.37
Cz06g02070	Violaxanthin de-epoxidase	<i>VDE</i>	-0.10	-0.28	-0.36
Cz02g29020	Long-chain-alcohol O-fatty-acyltransferase	<i>AAT?</i>	1.38	-1.04	0.36
OPP Pathway					
Cz06g12080	Glucose-6-phosphate 1-dehydrogenase	<i>G6PD1</i>	0.27	-0.45	-0.17
Cz03g12030	Glucose-6-phosphate 1-dehydrogenase	<i>G6PD2</i>	1.45	-1.46	-3.71
Cz04g18010	6-Phosphogluconolactonase	<i>PGLS1</i>	0.58	-0.05	0.55
Cz11g03250	6-Phosphogluconolactonase	<i>PGLS2</i>	-1.38	2.43	1.05
Cz05g06160	6-Phosphogluconate dehydrogenase	<i>6PGD</i>	-2.32	1.77	-0.53
Cz05g13260	Ribose 5-phosphate isomerase	<i>RPI1</i>	-5.36	5.45	0
Cz09g17220	Ribose 5-phosphate isomerase	<i>RPI2</i>	0.23	-0.57	-0.32
Cz05g11190	Ribulose-phosphate 3-epimerase	<i>RPE1</i>	2.33	-1.66	0.69
Cz04g31230	Ribulose-phosphate 3-epimerase	<i>RPE2</i>	1.15	-0.88	0.29
Cz14g07140	Ribulose-phosphate 3-epimerase	<i>RPE3</i>	2.33	-1.99	0.36
Cz03g04080	Transketolase	<i>TRK</i>	1.94	-2.01	-0.05
Cz06g25040	Transaldolase	<i>TAL1</i>	0.20	-0.82	-0.61
Cz04g12210	Transaldolase	<i>TAL2</i>	0.37	-0.15	0.24
Cz12g20290	Transaldolase	<i>TAL3</i>	-2.29	2.41	0.13
Glycolysis and Glucogenesis					
Cz13g07170	Hexokinase	<i>HK</i>	-0.73	1.32	0.60
Cz06g03010	Glucokinase	<i>GK</i>	-2.24	1.22	-1.00
Cz01g30020	Glucose-6-phosphate isomerase	<i>PGI</i>	-0.00	0.04	0.05
Cz09g25120	6-Phosphofructokinase	<i>PFK1</i>	-1.08	0.58	-0.48
Cz16g11260	6-Phosphofructokinase	<i>PFK2</i>	-1.36	2.49	1.14

Cz07g13120	6-Phosphofructokinase	<i>PFK3</i>	-1.54	0.93	-0.58
Cz04g03070	Fructose-1,6-bisphosphatase, class I	<i>FBP1</i>	2.52	-2.49	0.04
Cz05g01180	Fructose-1,6-bisphosphatase, class II	<i>FBP2</i>	0.71	0.91	1.63
Cz05g37140	Fructose-bisphosphate aldolase	<i>FBA1</i>	0.89	-1.05	-0.15
Cz06g07090	Fructose-bisphosphate aldolase	<i>FBA2</i>	0.11	-0.72	-0.59
Cz03g06050	Fructose-bisphosphate aldolase	<i>FBA3</i>	0.63	-0.46	0.19
Cz03g13070	Fructose-bisphosphate aldolase	<i>FBA4</i>	-2.42	1.66	-0.76
Cz06g17270	Triosephosphate isomerase	<i>TIM</i>	-0.28	0.18	-0.08
Cz05g34160	Glyceraldehyde 3-phosphate dehydrogenase (NAD)	<i>GAPDH</i>	-2.26	2.35	0.10
Cz16g19260	Phosphoglycerate kinase	<i>PGK</i>	0.38	-0.87	-0.47
Cz06g01110	Phosphoglycerate mutase, 2,3-bisphosphoglycerate-dependent	<i>PGAM1</i>	-2.81	2.28	-0.51
Cz09g03220	Phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent	<i>PGAM2</i>	0.15	-0.29	-0.12
Cz04g11130	Phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent	<i>PGAM3</i>	-3.66	3.38	-0.26
Cz08g21030	Phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent	<i>PGAM4</i>	-0.86	-0.23	1.08
Cz14g01200	Phosphoglycerate mutase	<i>PGAM5</i>	0.66	-0.99	-0.32
Cz14g08090	Phosphoglycerate mutase	<i>PGAM6</i>	0.56	-0.50	0.07
Cz01g38050	Phosphoglycerate mutase	<i>PGAM7</i>	-0.95	1.35	0.41
Cz05g10010	Enolase	<i>ENO</i>	-1.64	1.20	-0.42
UNPLg00362	Pyruvate kinase	<i>PK1</i>	0.38	-0.68	-0.28
Cz08g12170	Pyruvate kinase	<i>PK2</i>	1.75	-1.90	-0.13
Cz16g00040	Pyruvate kinase	<i>PK3</i>	-0.79	0.68	-0.10
Cz14g14130	Pyruvate kinase	<i>PK4</i>	1.75	-1.26	0.50
Cz04g17050	Pyruvate kinase	<i>PK5</i>	-1.41	1.54	0.15
Cz01g21060	Pyruvate kinase	<i>PK6</i>	-1.42	2.24	0.82
Cz15g09100	Pyruvate kinase	<i>PK7</i>	-0.28	0.28	0.02
Cz10g06190	Pyruvate kinase	<i>PK8</i>	0.63	-1.10	-0.45
Cz01g05160	Phosphoenolpyruvate carboxykinase (ATP)	<i>PEPCK</i>	0.30	1.83	2.15
Cz04g02090	Pyruvate carboxylase	<i>PYC</i>	0.47	-0.21	0.28
TCA					
Cz02g27080	Citrate synthase	<i>CIS1</i>	0.08	0.94	1.03
Cz02g12210	Citrate synthase	<i>CIS2</i>	-3.90	2.57	-1.32
Cz13g00140	Aconitate hydratase	<i>ACH</i>	0.34	-0.31	0.05
Cz11g28180	Isocitrate dehydrogenase, NAD-dependent	<i>IDH1</i>	-2.66	1.96	-0.68
Cz11g08120	Isocitrate dehydrogenase, NAD-dependent	<i>IDH2</i>	-1.57	0.86	-0.70
Cz12g16160	Isocitrate dehydrogenase, NADP-dependent	<i>IDH3</i>	0.31	-0.70	-0.38
Cz05g03220	2-Oxoglutarate dehydrogenase, E1	<i>OGDH1</i>	-1.08	0.37	-0.69
Cz01g28100	2-Oxoglutarate dehydrogenase, E1	<i>OGDH2</i>	1.41	-0.95	0.48

Cz02g11010	2-Oxoglutarate dehydrogenase, E2	<i>OGDH3</i>	-1.84	0.75	-1.07
Cz01g33150	Succinyl-CoA synthetase, alpha subunit	<i>SCSa</i>	-1.20	0.22	-0.97
Cz03g31200	Succinyl-CoA synthetase, beta subunit	<i>SCSb</i>	-2.19	1.33	-0.84
Cz15g13230	Succinate dehydrogenase (ubiquinone) iron-sulfur subunit	<i>SDH1</i>	-0.83	1.14	0.32
Cz07g14020	Succinate dehydrogenase (ubiquinone) cytochrome b560 subunit	<i>SDH2</i>	-1.79	1.76	-0.01
Cz07g14015	Succinate dehydrogenase (ubiquinone) membrane anchor subunit	<i>SDH3</i>	-1.01	0.91	-0.08
Cz07g04030	Fumarate hydratase, class I	<i>FHD</i>	0.07	0.05	0.14
Cz02g21340	Malate dehydrogenase (NAD)	<i>MDH</i>	-2.45	1.25	-1.18
Acetyl-CoA source					
Cz18g13050	Pyruvate dehydrogenase, E1 alpha	<i>PDHC1</i>	-0.38	0.23	-0.13
Cz07g25130	Pyruvate dehydrogenase, E1 beta	<i>PDHC2</i>	-1.66	1.39	-0.25
Cz03g08090	Pyruvate dehydrogenase, E1 alpha	<i>PDHC3</i>	-0.72	0.72	0.02
Cz01g37230	Pyruvate dehydrogenase, E1 beta	<i>PDHC4</i>	-1.57	1.76	0.21
Cz05g28130	Pyruvate dehydrogenase, E2 (dihydrolipoamide acetyltransferase)	<i>PDHC5</i>	-3.26	2.97	-0.27
Cz10g25060	Pyruvate dehydrogenase, E3 (dihydrolipoamide dehydrogenase)	<i>PDHC6</i>	-0.48	0.25	-0.21
Cz07g16120	Pyruvate dehydrogenase, E3 (dihydrolipoamide dehydrogenase)	<i>PDHC7</i>	-0.35	0.45	0.11
Cz01g33100	Pyruvate decarboxylase	<i>PDC</i>	-0.25	-0.48	-0.72
Cz11g04290	Aldehyde dehydrogenase (NAD ⁺)	<i>ALDH1</i>	-1.29	0.63	-0.64
Cz03g20090	Aldehyde dehydrogenase (NAD ⁺)	<i>ALDH2</i>	-0.59	-0.09	-0.66
Cz05g18170	Aldehyde dehydrogenase	<i>ALDH3</i>	0.10	-0.61	-0.49
Cz09g15060	Acetyl-CoA synthetase	<i>ACS1</i>	-1.05	0.50	-0.53
Cz12g10100	Acetyl-CoA synthetase	<i>ACS2</i>	0.87	-0.12	0.77

Table S2 Amino acid composition of heterotrophic cultured *C. zoefingiensis* after MP.

Amino acid	Composition (%)
Asp	9.97679
Ser	2.90932
Glu	14.18904
Gly	5.90862
His	2.96564
Arg	10.89968
Thr	2.01309
Ala	9.25401
Pro	6.02178
Cys	0
Tyr	0
Val	9.6075
Met	0
Lys	6.31571
Ile	5.60607
Leu	8.79234
Phe	5.54043