

Supplementary material

Sustainable production of lipids from cocoa fatty acid distillate fermentation driven by adaptive evolution in *Yarrowia lipolytica*

Naazneen Sofeo ^{a,*}, Toi Min Gin ^a, Ee En Qi Grace ^a, Ng Jing Yang ^a, Busran Coleen Toledo ^a, Benedict Ryan Lukito ^a, Aaron Thong ^a, Christian Hermansen ^a, Eric Charles Peterson ^{a,b}, Renata Glitsos ^a, Prakash Arumugam ^a

^a Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology, and Research (A*STAR), 31 Biopolis Way, Level 6, Nanos, Singapore 138669, Republic of Singapore

^b Institut National de la Recherche Scientifique – _Eau Terre Environnement (INRS-ETE), 490 Rue de la Couronne, Quebec City, QC G1K 9A9, Canada

*Email: naazneen_sofeo@sifbi.a-star.edu.sg

*Address: Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology, and Research (A*STAR), 31 Biopolis Way, Level 2, Nanos, Singapore 138669, Republic of Singapore

Table and Figure Legend:

Table A1: Parameters for quality of total RNA extracted and quality of RNA reads for mRNA sequencing for *Yarrowia lipolytica* strains.

Table A2: DEGs in EVO1 related to cell cycle, mitochondrial DNA and acid phosphatases and transporters at 10 and 24 h growth timepoints. ID is the YALI identifier of the gene, fold change refers to folds of up or down(-) regulation w.r.t the wild type, p-value(adj) refers to adjusted p-value and Desc refers to description of gene name or function.

Table A3: DEGs that were significant and common in both EVO1 and EVO3. Fold Change refers to folds of up or down(-) regulation w.r.t the wild type, p-value(adj) refers to adjusted p-value and Desc refers to description of gene name or function.

Figure A1: **A:**OD_{600nm} values for single colonies isolated from two evolving populations YL 697-5 and YL 697-20 for *Y. lipolytica* strains in ALE experiment. **B:** OD600nm values for different strains in YPD, CFAD 10 g/L and CFAD 20g/L concentrations at different timepoints of 10h and 24h. The values are from three biological replicates and are taken from the same cultures used for mRNA sequencing.

Figure A2: DEG characterization for EVO1 and EVO3 vs Wild type with p-value(adj)>0.05 and |log2FoldChange|>2. **A:** Number of differentially expressed genes (up- and down- regulated) at 10 h for EVO1. **B:** Number of differentially expressed genes (up- and down- regulated) at 24 h for EVO1. **C:** GO-term enrichment analysis for genes differentially expressed in Wild type vs EVO1 at 10 h. **D:** GO-term enrichment analysis for genes differentially expressed in Wild type vs EVO1 at 24 h. **E:** Number of differentially expressed genes (up- and down- regulated) at 10 h for EVO3. **F:** Number of differentially expressed genes (up- and down- regulated) at 24 h for EVO3. **G:** GO-term enrichment analysis for genes differentially expressed in Wild type vs EVO3 at 24 h.

Table A1:

Sample ID	Description	Conc (ng/ul)	RIN value	260/280	260/230	Raw reads	Clean reads	Err rate	Q30
WT_10_1	Wild type in CFAD 20g/L, 30C, 10 hr timepoint	648.9	9.6	2.18	2.5	27541492	27245650	0.02	94.88
WT_10_2	Wild type in CFAD 20g/L, 30C, 10 hr timepoint	237.5	9.6	2.24	2.41	27557012	27280228	0.02	95.12
WT_10_3	Wild type in CFAD 20g/L, 30C, 10 hr timepoint	487.9	9.8	2.22	2.51	25861136	25541418	0.02	94.71
53_10_1	697-5-3 in CFAD 20g/L, 30C, 10 hr timepoint	98.4	9.2	2.22	2.29	27501458	27287478	0.02	94.99
53_10_2	697-5-3 in CFAD 20g/L, 30C, 10 hr timepoint	97.1	8.9	2.23	2.33	25894528	25362140	0.02	94.35
53_10_3	697-5-3 in CFAD 20g/L, 30C, 10 hr timepoint	28.2	8.7	2.24	1.72	27475112	27134260	0.02	94.96
201_10_1	697-20-1 in CFAD 20g/L, 30C, 10 hr timepoint	490.1	10	2.23	2.48	24181444	23868276	0.02	94.58
201_10_2	697-20-1 in CFAD 20g/L, 30C, 10 hr timepoint	119.2	9.4	2.18	2.15	23601124	23327250	0.02	95.1
201_10_3	697-20-1 in CFAD 20g/L, 30C, 10 hr timepoint	158.7	9.9	2.23	2.32	27744006	27411652	0.02	95.07
WT_24_1	Wild type in CFAD 20g/L, 30C, 24 hr timepoint	259.8	9.9	2.14	2.37	23673544	23380656	0.02	95.07
WT_24_2	Wild type in CFAD 20g/L, 30C, 24 hr timepoint	94.6	10	2.08	2.39	25277472	24958680	0.02	94.62
WT_24_3	Wild type in CFAD 20g/L, 30C, 24 hr timepoint	213.7	10	2.15	2.38	24034268	23756776	0.02	94.91
53_24_1	697-5-3 in CFAD 20g/L, 30C, 24 hr timepoint	230.7	9.9	2.22	2.56	26949894	26621476	0.02	94.81
53_24_2	697-5-3 in CFAD 20g/L, 30C, 24 hr timepoint	213	9.7	2.2	2.5	27972712	27600264	0.02	94.69
53_24_3	697-5-3 in CFAD 20g/L, 30C, 24 hr timepoint	215.7	9.8	2.22	2.54	21951724	21548954	0.02	94.78
201_24_1	697-20-1 in CFAD 20g/L, 30C, 24 hr timepoint	348.3	10	2.24	2.53	23289590	23036800	0.02	94.67
201_24_2	697-20-1 in CFAD 20g/L, 30C, 24 hr timepoint	182.9	10	2.21	2.47	22274774	21969780	0.02	94.74
201_24_3	697-20-1 in CFAD 20g/L, 30C, 24 hr timepoint	159.2	9.9	2.21	2.59	23172480	22908092	0.02	94.67

Table A2

CELL CYCLE GENES				
Gene	ID	Fold change	p-value(adj)	Desc
10 h				
Dad3	YALI0B17303	4.2	2.99E-08	Dam1 complex
Dam1	YALI0F05236	2.2	0.000151418	Dam1 complex
Spc34	YALI0F15895	1.6	0.001120516	Dam1 complex
Mis13	YALI0E03498	2.4	0.003346167	Dam1 complex
Duo1	YALI0C18975	3.1	0.003857189	Dam1 complex
Dad4	YALI0C00715	1.9	0.006542068	Dam1 complex
Fta4	YALI0E32659	3.0	0.013447723	Component of Sim4 subunit
Spc19	YALI0F27335	2.6	0.018848447	Dam1 complex
Nnf1	YALI0C23925	2.1	0.034144077	Component of the NMS (Ndc80-MIND-Spc7) super complex
Cnl2/NKP2 family protein	YALI0E26521	2.6	0.000188008	Cnl2/NKP2 family protein
MCM21	YALI0C24123	1.6	0.018513834	Component of the kinetochore sub-complex COMA
NSL1	YALI0B04466	1.9	0.028302224	Part of Mis12 complex
MTW1	YALI0F06138	2.2	0.043520823	Part of Mis12/mtw1 complex
IME2	YALI0F03113	1.8	2.87E-05	IME2 Meiosis induction protein kinase
ALP6	YALI0C09757	1.8	0.010881654	ALP6 Spindle pole body component
RAD53	YALI0F27093	2.9	0.002054906	Cell cycle checkpoint kinase
G1/S-specific cyclin CCN1	YALI0C13926	2.7	0.045305171	Cyclin E1
EF-Tu	YALI0A20152	-8.4	0.002565809	Transcriptional elongation factor
RNY1	YALI0F25839	30.0	0.000195011	tRNA Rnase, vacuolar and cytosolic
24 h				
Spc34	YALI0F15895	-3.2	0.003987831	Dam1 complex
MITOCHONDRIAL GENES				
Gene	ID	Fold change	p-value(adj)	Desc
10 h				
atp9	atp9	20.1	0.030250414	ATP synthase subunit 9
nd6	nd6	18.2	0.001438349	NADH-ubiquinone oxidoreductase chain 6
nd3	nd3	14.9	0.030597513	NADH-ubiquinone oxidoreductase chain 3
nd1	nd1	10.7	0.022798588	NADH-ubiquinone oxidoreductase chain 1
cox3	cox3	9.6	0.006320644	Cytochrome c oxidase subunit 3
cob	cob	9.4	0.041133947	Cytochrome b
cox1	cox1	9.1	0.027516554	Cytochrome c oxidase subunit 1
nd4	nd4	8.7	0.007561711	NADH-ubiquinone oxidoreductase chain 4
atp6	atp6	7.6	0.003298218	ATP synthase subunit a
nd5	nd5	7.2	0.00115383	NADH-ubiquinone oxidoreductase chain 5
cox2	cox2	5.2	2.84E-05	Cytochrome c oxidase subunit 2
YalifMr30	YalifMr30	23.6	0.00130968	23S ribosomal RNA
YalifMr31	YalifMr31	13.4	0.04968897	16S ribosomal RNA
RPR1	RPR1	5.4	7.68E-05	RNase P RNA
SCR1	SCR1	4.8	0.0003514	SRP RNA
SNR17	SNR17	4.1	3.26E-05	U3 RNA
SNR17	SNR17	3.0	0.00321214	U3 RNA
SNR17	SNR17	2.8	0.00125061	U3 RNA
ACID PHOSPHATASES AND TRANSPORTERS				
Gene	ID	Fold change	p-value(adj)	Desc
10 h				
PHO89	YALI0E23859	58.1	1.40E-39	Phosphate transporter
MEP2	YALI0E27203	-20.1	3.86E-17	Ammonium transporter. Works during nitrogen starvation
YALI0D08382	YALI0D08382	14.7	8.13E-16	Plasma membrane permease for glycerol-PC and PI. Similar to GIT1
YALI0E35222	YALI0E35222	4.9	0.000851	Acid phosphatase
YALI0E00110	YALI0E00110	4.4	9.87E-20	
YALI0F25773	YALI0F25773	4.2	0.00018352	
YALI0C19866	YALI0C19866	40.5	1.66E-19	

YALI0D03465	YALI0D03465	12.5	2.42E-10	PHO2 Acid phosphatase, Similar to survival protein E.
YALI0A12573	YALI0A12573	9.9	5.76E-13	
PHO2/SurE	YALI0C19866	40.5	1.66E-19	
PHO2/SurE	YALI0D03465	12.5	2.42E-10	
24 h				
CAF5 like	YALI0F25597	-15.4	0.02168265	Caffeine efflux pump
ZRT1 like	YALI0D00759	727.5	8.27E-29	Zinc transporter
TNA1	YALI0F28193	179.5	1.92E-20	High affinity nicotinic acid plasma membrane permease
cnsO like	YALI0E27247	10.7	0.01753686	High affinity nicotinic acid plasma membrane permease
STL1	YALI0C04730	7.1	0.0035931	Sugar transporter, reported to be expressed at low nitrogen
MEP2	YALI0E27203	-53.8	0.01246818	Ammonium transporter. Works during nitrogen starvation
UP2	YALI0F04598	-18.6	0.00023669	Putative extracellular fatty acid binding
PF01490	YALI0C12100	-73.9	5.54E-05	Transmembrane amino acid transporter protein
GAP1/GAP2	YALI0B19492	32.1	4.79E-09	General amino acid transporter like
DUR3	YALI0B04202	-15.3	0.02510543	Urea active transporter
MUP1	YALI0F25795	-87.6	0.00060279	High-affinity methionine permease

Table A3

		EVO1		EVO3		
Gene	ID	Fold change	p-value(adj)	Fold change	p-value(adj)	Desc
10 h						
RGT1	YALI0C07821	4.0	3.27E-05	7.2	0.00124866	glucose transport transcription regulator
PHO89	YALI0E23859	58.1	1.40E-39	22.3	0.00467382	Phosphate transporter
YALI0A12573	YALI0A12573	9.9	5.76E-13	6.7	0.00028432	Related to thiamine synthesis
FAD1/OLE1	YALI0C05951	2.6	7.99E-05	3.9	0.0001184	Desaturase
LYC1	YALI0E05533	2.1	2.30E-05	2.7	0.000366	Lys acetyl-transferase
lip15	YALI0E11561	-3.0	0.00263443	-4.5	0.01883496	Intracellular lipase
YALI0B07117	YALI0B07117	-3.9	4.81E-05	-4.4	0.00351723	Erythrose reductase homolog

Figure A1:

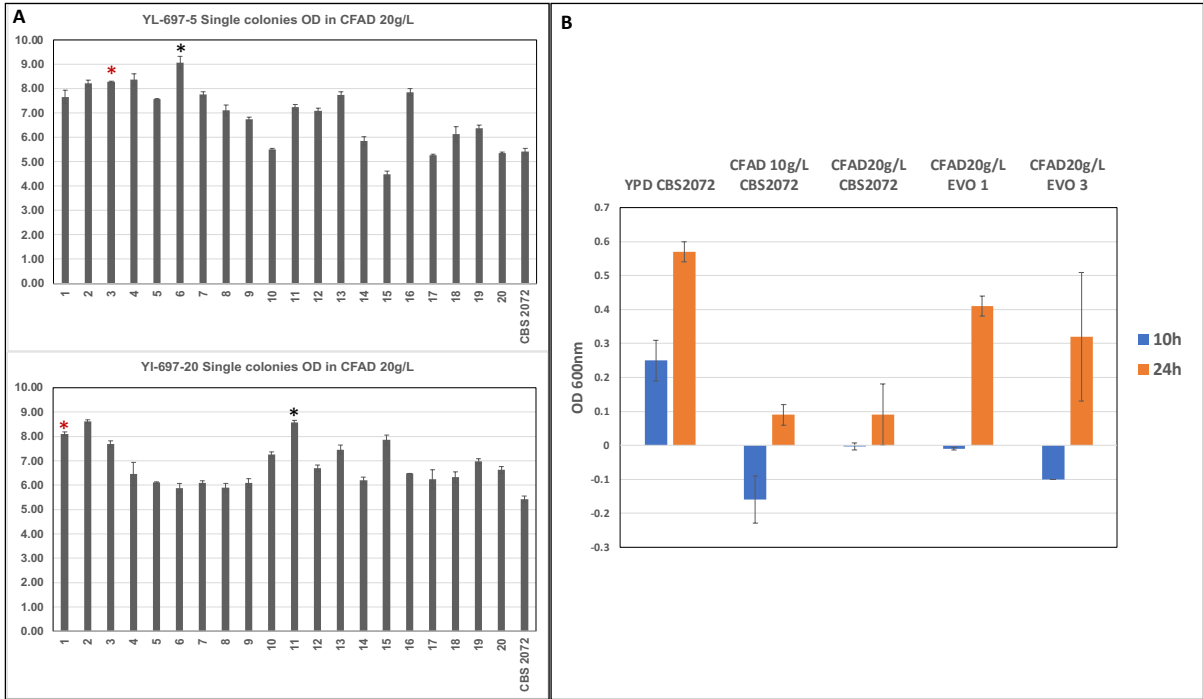


Figure A2:

