

Strain-specific diversity in lipid and carotenoid profiles of *Rhodotorula toruloides*

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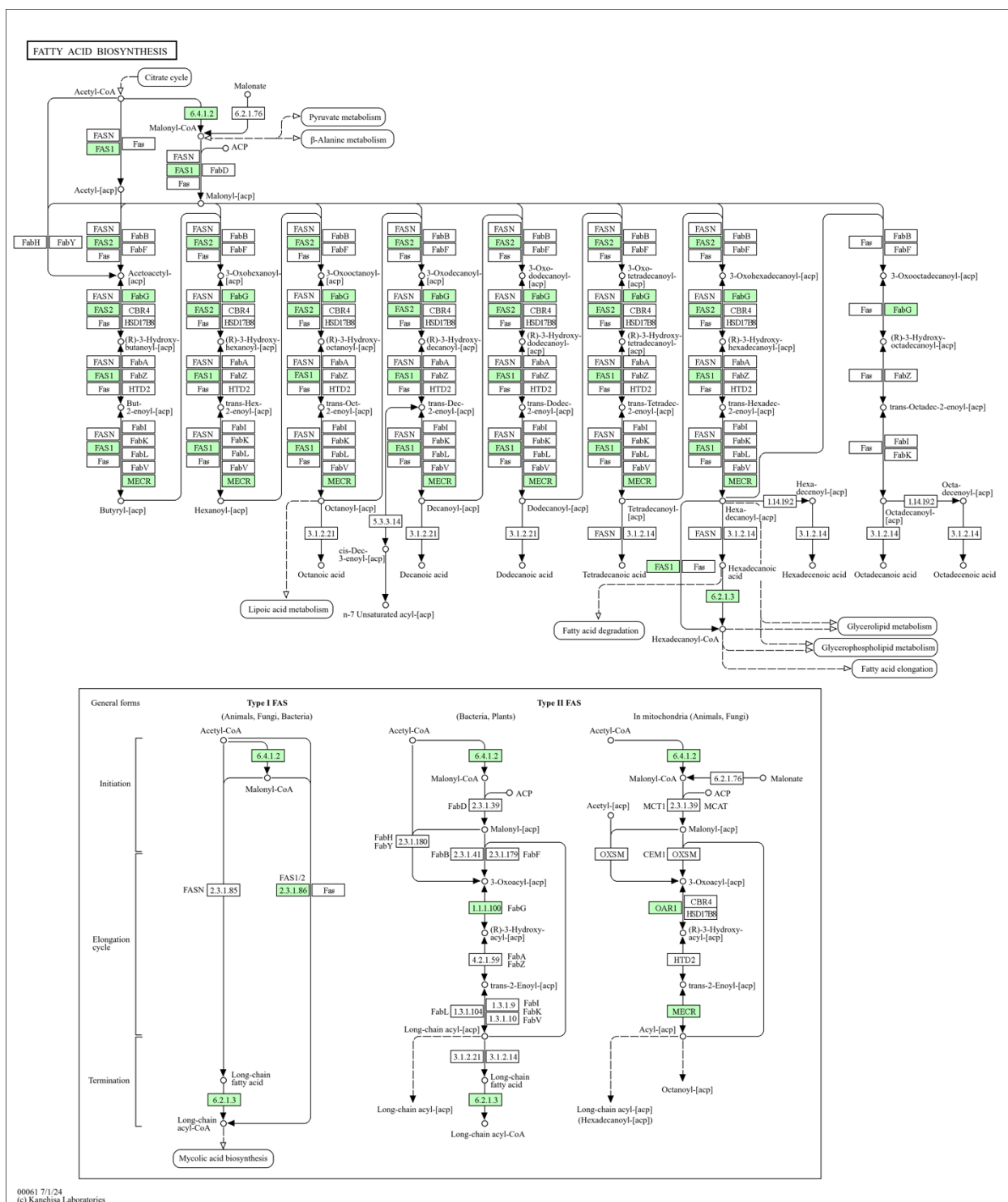


Fig. S1: Fatty acid biosynthesis. Fatty acid synthesis pathway in CBS 14 (green). Source: KEGG pathway database.

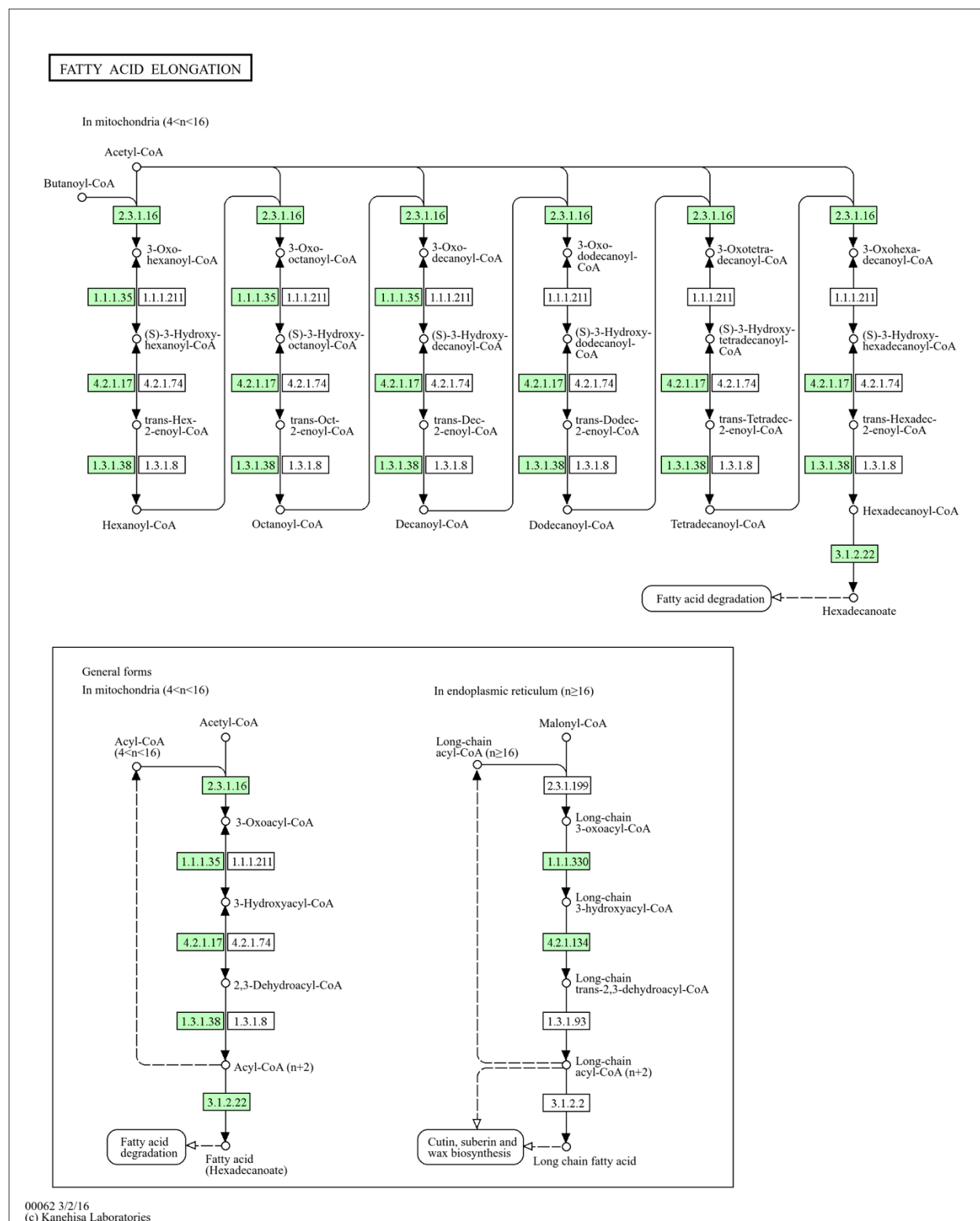


Fig. S2: Fatty acid biosynthesis. Fatty acid elongation in CBS 14 (green). Source: KEGG pathway database.

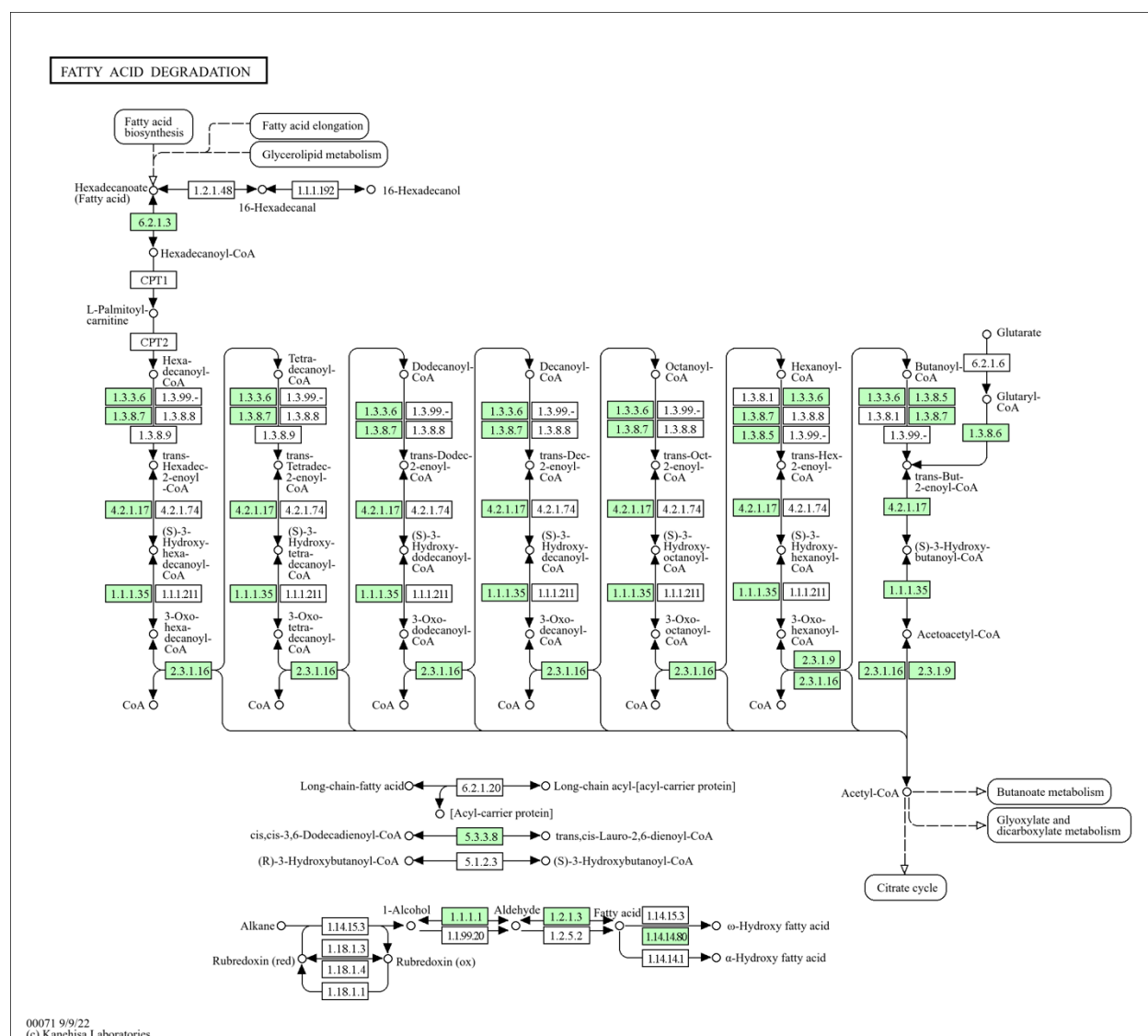


Fig. S3: Fatty acid degradation in CBS 14 (green). Source: KEGG pathway database.

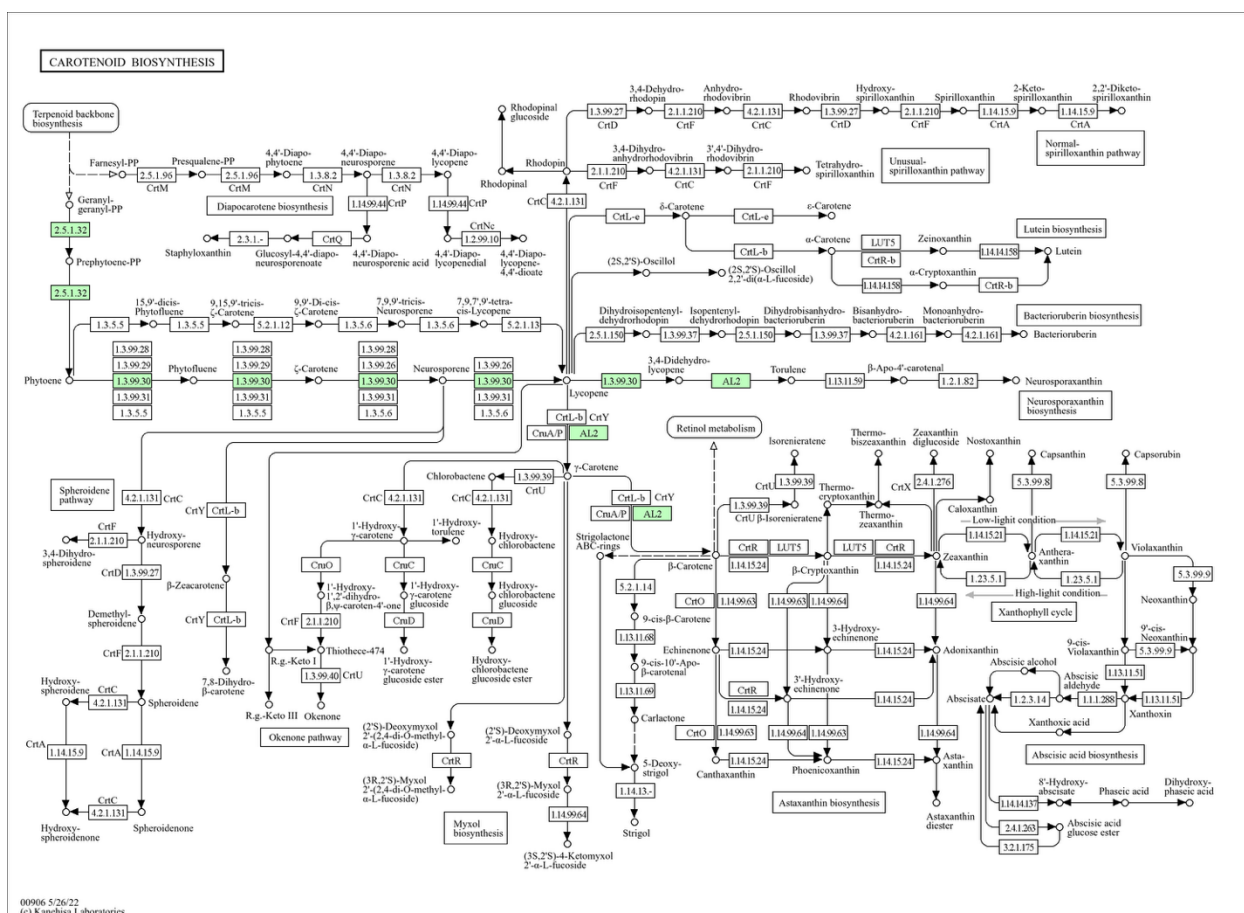


Fig. S4: Carotenoid biosynthesis in CBS 14 (green). Source: KEGG pathway database.

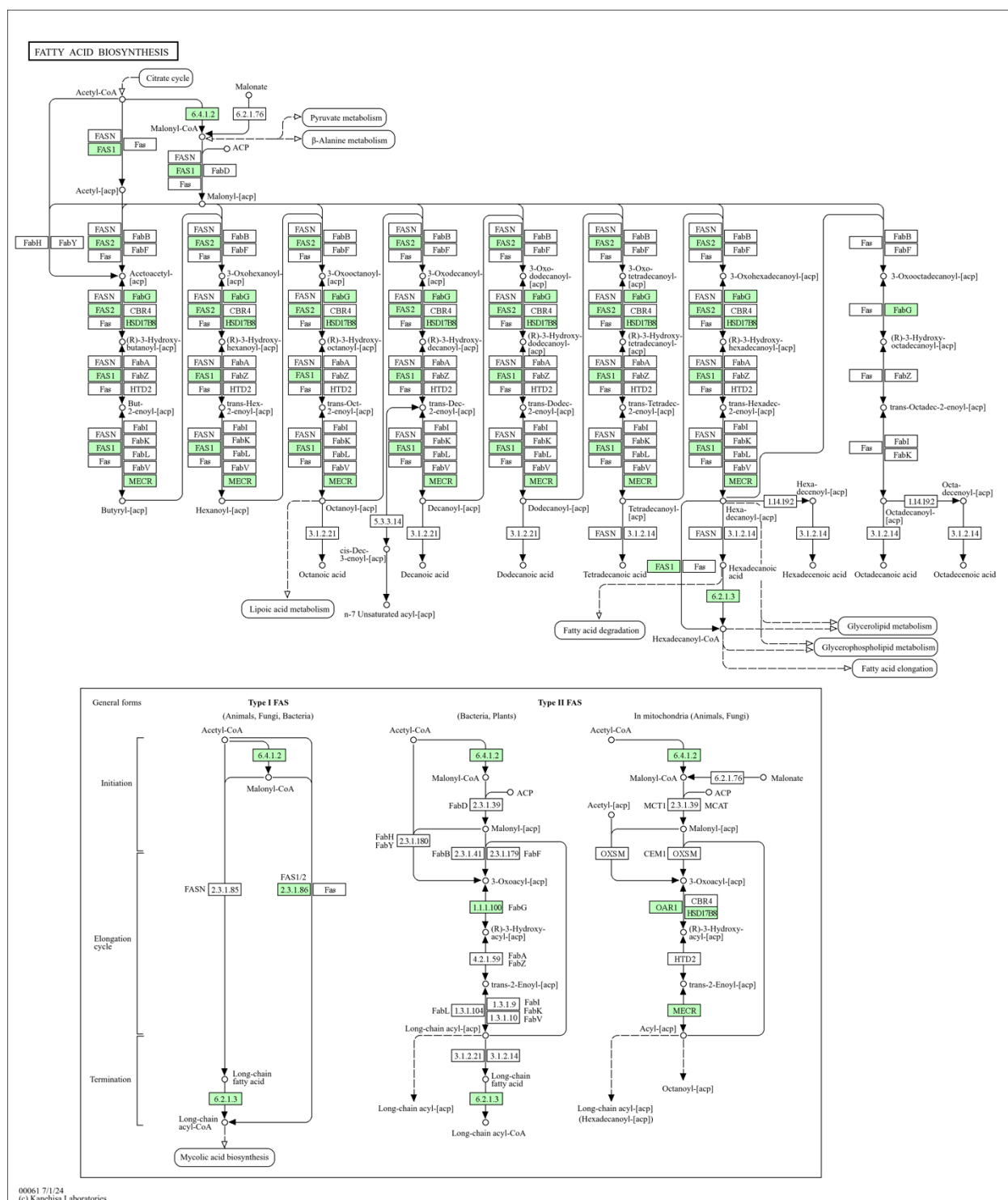


Fig. S5: Fatty acid biosynthesis. Fatty acid synthesis pathway in CBS 6016 (green). Source: KEGG pathway database.

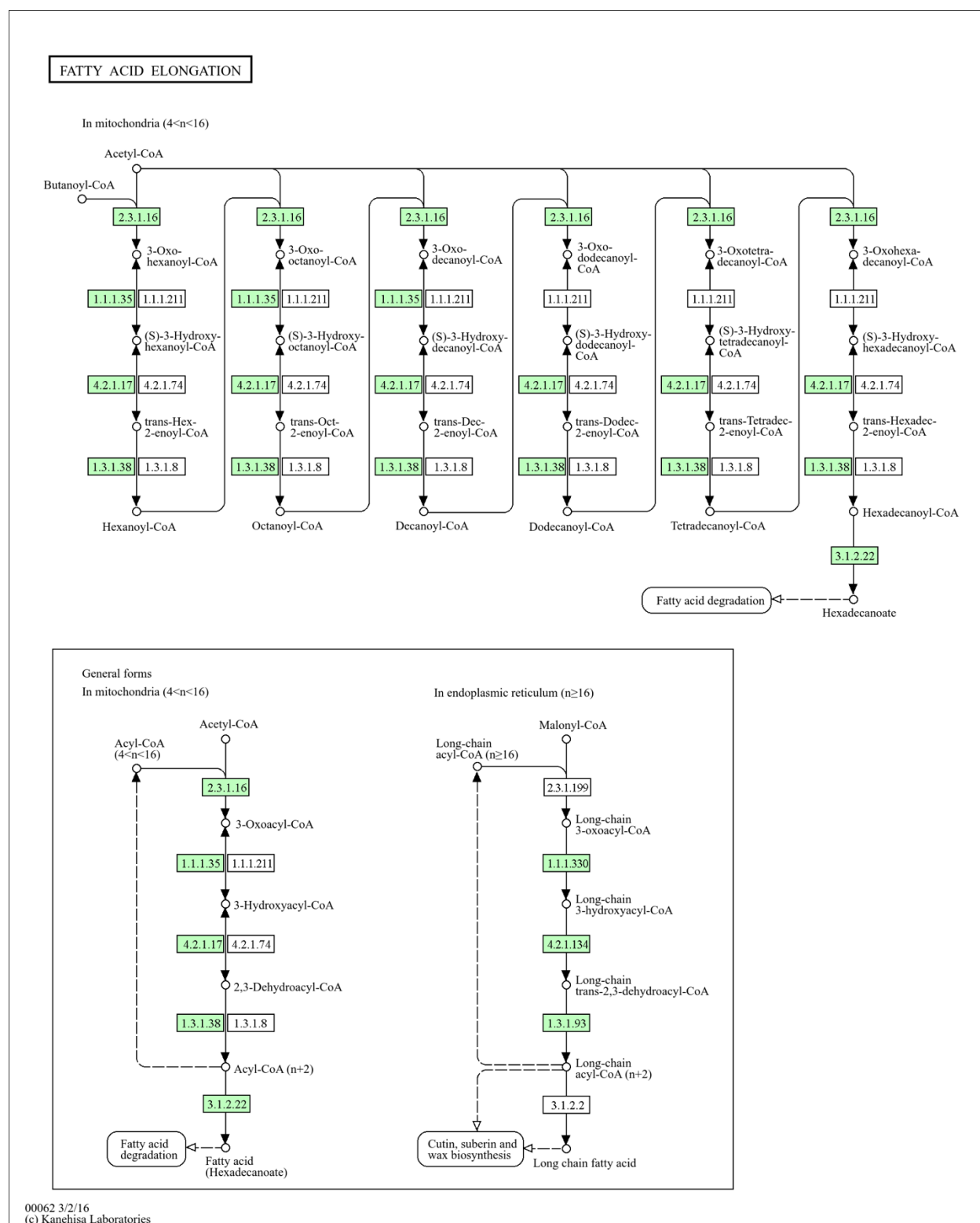


Fig. S6: Fatty acid biosynthesis. Fatty acid elongation in CBS 6016 (green). Source: KEGG pathway database.

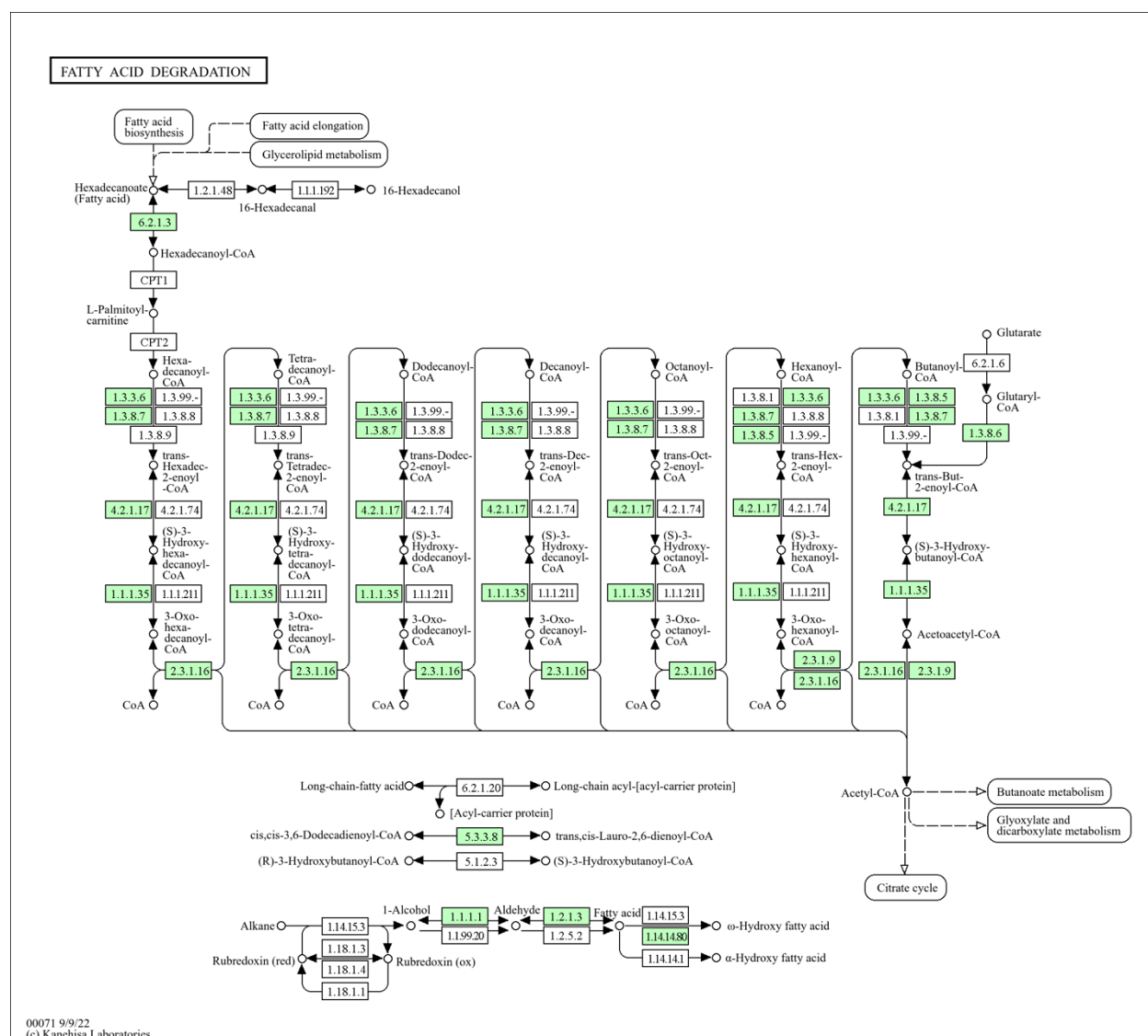


Fig. S7: Fatty acid degradation in CBS 6016 (green). Source: KEGG pathway database.

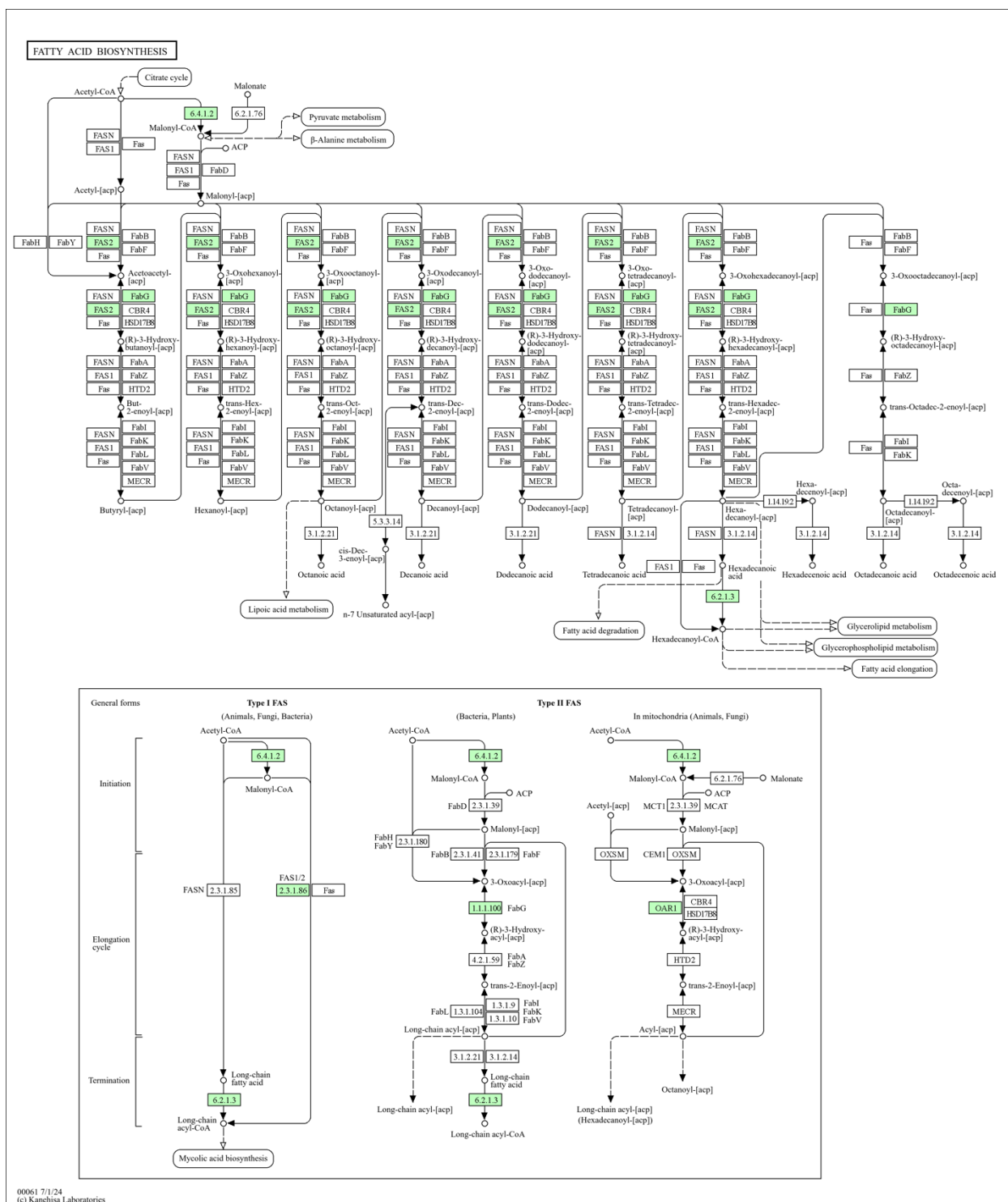


Fig. S9: Fatty acid biosynthesis. Fatty acid synthesis pathway in CBS 349 (green). Source: KEGG pathway database.

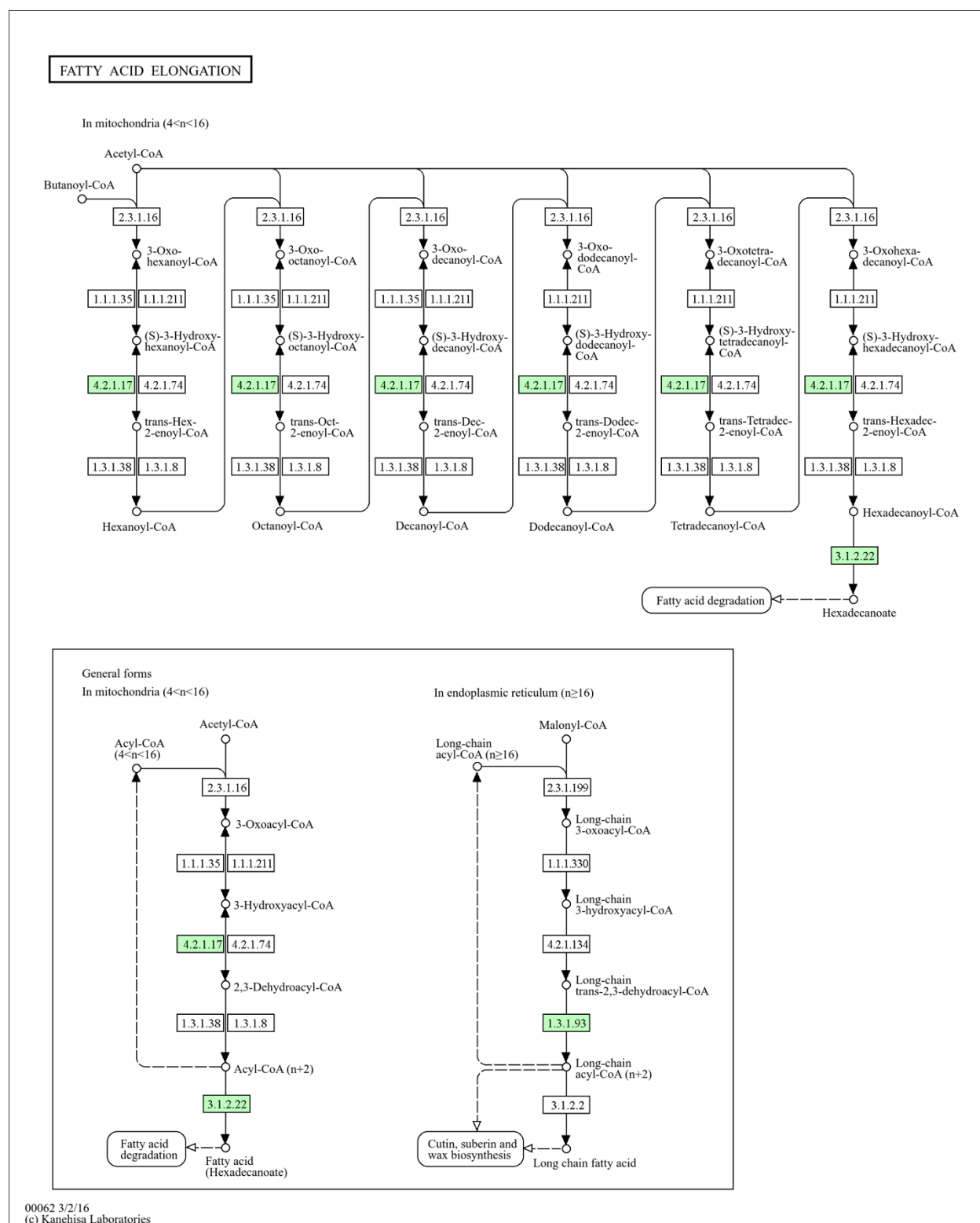


Fig. S10: Fatty acid biosynthesis. Fatty acid elongation in CBS 349 (green). Source: KEGG pathway database.

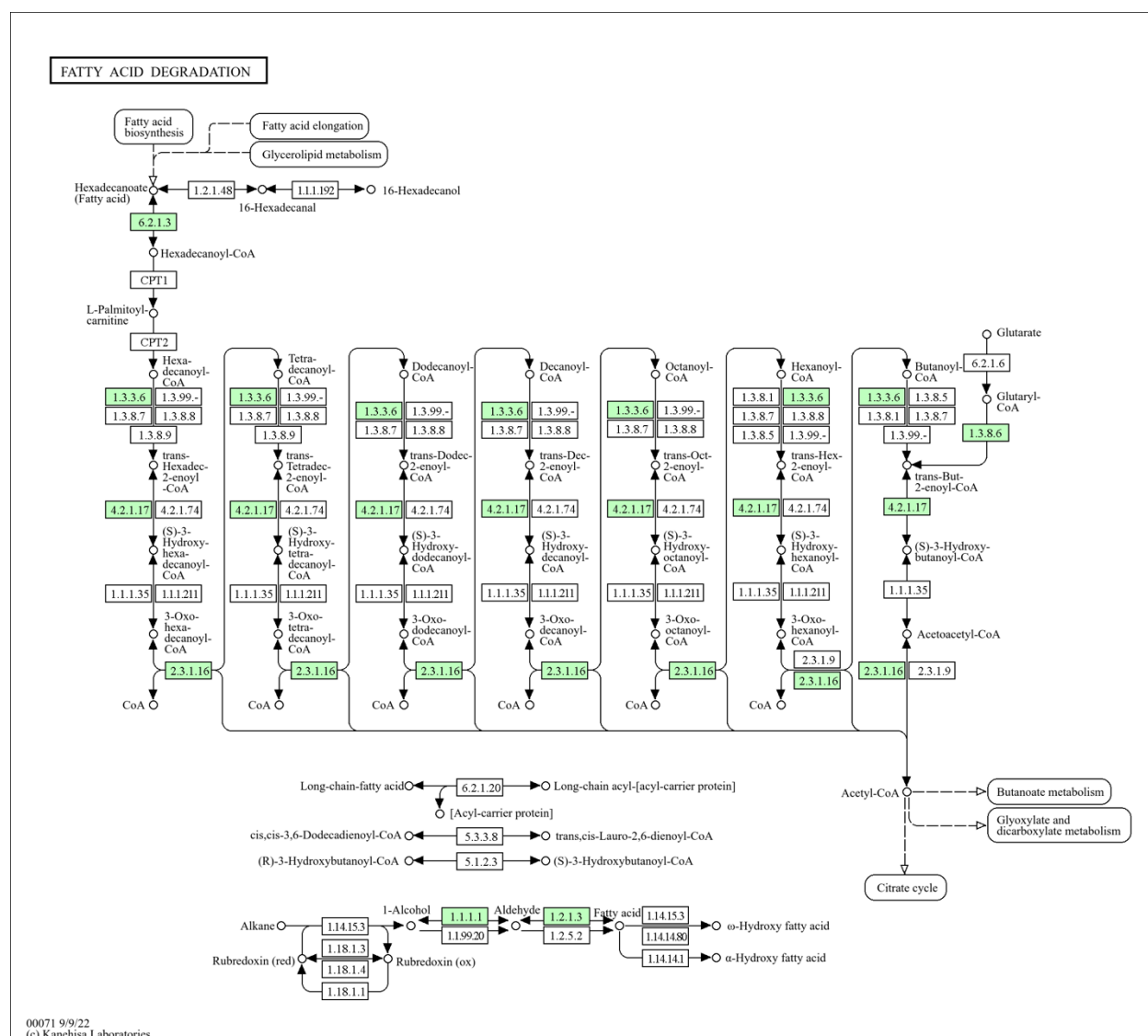


Fig. S11: Fatty acid degradation in CBS 349 (green). Source: KEGG pathway database.

Table S1: Protein domains and signatures predicted for FAS1 in *Rhodotorula toruloides* CBS349, *Rhodotorula toruloides* CBS14, and *Saccharomyces cerevisiae*. InterProScan (reference: Blum M et al. InterPro: the protein sequence classification resource. Nucleic Acids Research. 2024, doi: 10.1093/nar/gkae1082). Search for FAS1 performed on the 20250625.

Accession (InterPro)	Short name	Name	<i>S. cerevisiae</i> 2051 aa	CBS14 (CBS14_11041) 1328 aa	CBS349 (CBS349_2707) 851 aa
IPR050830	Fungal_FAS	Fungal fatty acid synthase	x	x	x
IPR002539	MaoC-like_dom	MaoC-like dehydratase domain	x	In FAS2	In FAS2
IPR029069	HotDog_dom_sf	HotDog domain superfamily	x	In FAS2	In FAS2
IPR040883	FAS_meander	Fatty acid synthase, meander beta sheet domain	x	x	In FAS2
IPR016035	Acyl_Trfase/lysoPLipase	Acyl transferase/acyl hydrolase/lysophospholipase	x	x	x
IPR001227	Ac transferase_dom_sf	Acyl transferase domain superfamily	x	x	x
IPR003965	Fatty acid synthase	Fatty acid synthase	x	x	x
IPR016452	Fas1/AflB-like	Fatty acid synthase beta subunit AflB /Fas1-like, fungi	x	In FAS2 (IPR003965)	In FAS2 (IPR003965)
IPR039569	FAS1-like_DH_region	FAS1-like, dehydratase domain region	x	In FAS2	In FAS2
IPR013785	Aldolase_TIM	Aldolase-type TIM barrel	x	x	x
IPR032088	SAT	Starter unit:ACP transacylase, SAT domain	x	x	x
IPR013565	Fas1/AflB-like_central	Fatty acid synthase beta subunit AflB /Fas1-like, central domain	x	x	x
IPR014043	Acyl_transferase_dom	Acyl transferase domain	x	In FAS2	In FAS2
IPR041099	FAS1_N	Fatty acid synthase subunit beta, N-terminal domain	x	x	-

Table S2: Protein domains and signatures predicted for FAS2 of *Saccharomyces cerevisiae* and *Rhodotorula toruloides* CBS 349. InterProScan (reference: Blum M et al. InterPro: the protein sequence classification resource. Nucleic Acids Research. 2024, doi: 10.1093/nar/gkac1082). Search for FAS2 performed on the 20250625.

Accession (InterPro)	Short name	Name	<i>S. cerevisiae</i> 1887 aa	CBS349 (CBS349_2830) 2898 aa
IPR016039	<u>Thiolase-like</u>	Thiolase-like	x	x
IPR014043	<u>Acyl transferase dom</u>	<u>Acyl transferase domain</u>	In FAS1	x
IPR003965	<u>Fatty acid synthase</u>	<u>Fatty acid synthase</u>	In FAS1	x
IPR026025	<u>FAS_alpha_yeast</u>	<u>Fatty acid synthase alpha subunit, yeast</u>	x	-
IPR004568	<u>Pantetheine-prot Trfase dom</u>	<u>Phosphopantetheine-protein transferase domain</u>	x	x
IPR041550	<u>FASI_helical</u>	<u>Fatty acid synthase type I, helical</u>	x	x
IPR039569	<u>FAS1-like_DH_region</u>	<u>FAS1-like, dehydratase domain region</u>	In FAS1	x
IPR002582	<u>ACPS</u>	<u>Holo-[acyl carrier protein] synthase</u>	x	x
IPR020841	<u>PKS_Beta-ketoAc synthase dom</u>	<u>Polyketide synthase, beta-ketoacyl synthase domain</u>	x	x
IPR001227	<u>Ac transferase dom_sf</u>	<u>Acyl transferase domain superfamily</u>	In FAS1	x
IPR016035	<u>Acyl Trfase/lysoPLipase</u>	<u>Acyl transferase/acyl hydrolase/lysophospholipase</u>	x	x
IPR040899	<u>Fas_alpha_ACP</u>	<u>Fatty acid synthase subunit alpha, acyl carrier domain</u>	x	x
IPR009081	<u>PP-bd_ACP</u>	<u>Phosphopantetheine binding ACP domain</u>	x	x
IPR037143	<u>4-PPantetheinyl Trfase dom_sf</u>	<u>4'-phosphopantetheinyl transferase domain superfamily</u>	x	x
IPR047224	<u>FAS_alpha_su_C</u>	<u>Fatty acid synthase subunit alpha-like, C-terminal</u>	x	x
IPR050830	<u>Fungal_FAS</u>	<u>Fungal fatty acid synthase</u>	x	x
IPR029069	<u>HotDog_dom_sf</u>	<u>HotDog domain superfamily</u>	In FAS1	x
IPR014030	<u>Ketoacyl synth_N</u>	<u>Beta-ketoacyl synthase-like, N-terminal</u>	x	x
IPR014031	<u>Ketoacyl synth_C</u>	<u>Beta-ketoacyl synthase, C-terminal</u>	x	-
IPR008278	<u>4-PPantetheinyl Trfase dom</u>	<u>4'-phosphopantetheinyl transferase domain</u>	x	x
IPR018201	<u>Ketoacyl synth_AS</u>	<u>Beta-ketoacyl synthase, active site</u>	x	x

IPR036291	<u>NAD(P)-bd_dom_sf</u>	<u>NAD(P)-binding domain superfamily</u>	x	x
IPR040883	<u>FAS_meander</u>	<u>Fatty acid synthase, meander beta sheet domain</u>	In FAS1	x
IPR002539	<u>MaoC-like_dom</u>	<u>MaoC-like dehydratase domain</u>	In FAS1	x

Same as Table S1: Pairwise amino acid sequence identity of key proteins in lipid and carotenoid metabolism. This matrix illustrates the percentage identity of deduced amino acid sequences for genes involved in lipid and carotenoid biosynthesis across the *Rhodotorula toruloides* strains CBS 14, CBS 349, and CBS 6016. Rows and columns represent homologs from different strains or the same strains. The values indicate pairwise identity (%) between sequences. Green indicates 100% identity. FAS1/FAS2: Fatty acid synthase subunits (EC 2.3.1.86 and 2.3.1.85), ACC: Acetyl-CoA carboxylase (EC 6.4.1.2, ACACA), malonyl-CoA forming, FAA: Long-chain fatty acid CoA ligases (EC 6.2.1.3), facilitating fatty acid activation for degradation, AL1: Phytoene desaturase (EC 1.3.99.30), part of the carotenoid synthesis pathway up to lycopene. AL2: Bifunctional phytoene synthase (EC 2.5.1.32), involved in the synthesis of phytoene and further to toluene and torularhodin.

	%identity of deduced amino acid sequences							
FAS2	CBS14_7852 (2981 aa)	CBS14_7853 (2637 aa)	CBS349_2830 (2900 aa)	CBS349_2834 (1183 aa)	CRS6016_160 (2896 aa)	CRS6016_10604 (2981 aa)	CRS6016_10607 (2698 aa)	CRS6016_10608 (2976 aa)
CBS14_7852 (2981 aa)		100.0	94.9	99.4	94.8	100.0	100.0	100.0
CBS14_7853 (2637 aa)	100.0		97.9	99.4	97.9	100.0	100.0	100.0
CBS349_2830 (2900 aa)	94.9	97.9		98.2	100.0	94.9	97.1	94.9
CBS349_2834 (1183 aa)	99.4	99.4	98.2		98.2	99.4	99.4	99.4
CRS6016_160 (2896 aa)	94.8	97.9	100.0	98.2		94.8	97.1	94.8
CRS6016_10604 (2981 aa)	100.0	100.0	94.9	99.4	94.8		100.0	100.0
CRS6016_10607 (2698 aa)	100.0	100.0	97.1	99.4	97.1	100.0		100.0
CRS6016_10608 (2976 aa)	100.0	100.0	94.9	99.4	94.8	100.0	100.0	
FAS1	CBS14_11040 (1267 aa)	CBS14_11041 (1329 aa)	CBS349_2707 (850 aa)	CRS6016_255 (1282 aa)	CRS6016_18410 (1267 aa)	CRS6016_18411 (1334 aa)	CRS6016_18412 (1329 aa)	
CBS14_11040 (1267 aa)		100.0	98.8	98.3	100.0	100.0	100.0	
CBS14_11041 (1329 aa)	100.0		98.8	98.3	100.0	100.0	100.0	
CBS349_2707 (850 aa)	98.8	98.8		99.9	98.8	98.8	98.8	
CRS6016_255 (1282 aa)	98.3	98.3	99.9		98.3	98.3	98.3	
CRS6016_18410 (1267 aa)	100.0	100.0	98.8	98.3		100.0	100.0	
CRS6016_18411 (1334 aa)	100.0	100.0	98.8	98.3	100.0		100.0	
CRS6016_18412 (1329 aa)	100.0	100.0	98.8	98.3	100.0	100.0		
ACC	CBS14_11075 (2233 aa)	CRS349_2674 (1439 aa)	CRS6016_281 (2266 aa)	CRS6016_18444 (2233 aa)	CRS6016_18445 (1678 aa)			
CBS14_11075 (2233 aa)		99.5	99.4	100.0	100.0			
CRS349_2674 (1439 aa)	99.5		99.9	99.5	99.7			
CRS6016_281 (2266 aa)	99.4	99.9		99.4	99.3			
CRS6016_18444 (2233 aa)	100.0	99.5	99.4		100.0			
CRS6016_18445 (1678 aa)	100.0	99.5	99.3	100.0				
FAA	CBS14_3269 (698 aa)	CBS14_7230 (705 aa)	CRS6016_2924 (705 aa)	CRS6016_7018 (698 aa)	CRS6016_12861 (639 aa)			
CBS14_3269 (698 aa)		53.0	53.0	100.0	89.8			
CBS14_7230 (705 aa)	53.0		100.0	53.0	50.1			
CRS6016_2924 (705 aa)	53.0	100.0		53.0	50.1			
CRS6016_7018 (698 aa)	100.0	53.0	53.0		89.8			
CRS6016_12861 (639 aa)	89.8	50.1	50.1	89.8				
AL1	CBS14_2943 (567 aa)	CBS14_2944 (210 aa)	CBS349_2617 (543 aa)	CRS6016_331 (506 aa)	CRS6016_16131 (561 aa)	CRS6016_16132 (492 aa)	CRS6016_16133 (262 aa)	
CBS14_2943 (567 aa)		96.7	95.3	95.0	100.0	98.4	97.3	
CBS14_2944 (210 aa)	96.7		86.7	86.7	96.7	100.0	100.0	
CBS349_2617 (543 aa)	95.3	86.7		100.0	95.3	93.1	89.3	
CRS6016_331 (506 aa)	95.0	86.7	100.0		95.0	92.5	89.3	
CRS6016_16131 (561 aa)	100.0	96.7	95.3	95.0		98.4	97.3	
CRS6016_16132 (492 aa)	98.4	100.0	93.1	92.5	98.4		100.0	
CRS6016_16133 (262 aa)	97.3	100.0	89.3	89.3	97.3	100.0		
AL2	CBS14_2940 (612 aa)	CRS6016_327 (599 aa)	CRS6016_16127 (612 aa)					
CBS14_2940 (612 aa)		95.688	100					
CRS6016_327 (599 aa)	95.688		95.688					
CRS6016_16127 (612 aa)	100	95.688						