Additional file 1

High-throughput screening of Mucoromycota fungi for production of low- and high-value lipids

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Table of contents: Page

Table S1 Mucoromycota strains showing the 10 highest biomass concentration (g/L), total lipid content of the biomass (%), total lipid concentration (g/L medium), GLA and ARA production (% of total fatty acids, g/L medium)

Strain	Biomass (g/L)	Strain	Total lipid (%)	Strain	Total lipid (g/L)
Umbelopsis vinacea UBOCC-A-101347	25.6	Absidia glauca CCM 451	47.2	Cunninghamella blakesleeana CCM F-705	8.3
Cunninghamella blakesleeana CCM F-705	22.6	Mortierella alpina ATCC 32222	44.5	Umbelopsis vinacea CCM F-539	7.9
Umbelopsis vinacea CCM F-539	22.6	Absidia glauca CCM 450	39.1	Umbelopsis vinacea UBOCC-A-101347	7.0
Cunninghamella blakesleeana VKM F-993	20.0	Cunninghamella echinulata VKM F-439	37.2	Absidia glauca CCM 450	6.5
Umbelopsis isabellina VKM F-525	19.6	Cunninghamella blakesleeana CCM F-705	36.7	Absidia glauca CCM 451	6.2
Lichtheimia corymbifera CCM 8077	17.8	Cunninghamella echinulata VKM F-470	36.5	Umbelopsis isabellina VKM F-525	6.0
Absidia coerulea CCM 8230	17.5	Absidia cylindrospora VKM F-1632	36.5	Absidia coerulea CCM 8230	5.3
Absidia glauca CCM 450	16.6	Absidia coerulea VKM F-627	35.6	Cunninghamella blakesleeana VKM F-993	5.3
Umbelopsis ramanniana VKM F-502	15.3	Absidia glauca UBOCC-A-101330	34.8	Mortierella gemmifera VKM F-1631	5.1
Mucor circinelloides VI 04473	15.1	Mortierella gemmifera VKM F-1651	34.8	Cunninghamella echinulata VKM F-470	4.9

Strain	GLA (% total FAs)	Strain	ARA (% total FAs)
Mucor fragilis UBOCC-A-109196	24.5	Mortierella alpina ATCC 32222	41.1
Cunninghamella echinulata VKM F-470	24.0	Mortierella hyalina VKM F-1854	26.7
Mucor mucedo UBOCC-A-101353	21.5	Mortierella alpina UBOCC-A-112046	24.6
Mucor racemosus UBOCC-A-111127	20.9	Mortierella alpina UBOCC-A-112047	20.0
Mucor flavus VKM F-1110	20.9	Mortierella gamsii VKM F-1529	18.9
Mucor lanceolatus UBOCC-A-101355	20.9	Mortierella gamsii VKM F-1641	17.9
Rhizopus stolonifer VKM F-400	20.3	Mortierella globulifera VKM F-1408	16.1
Cunninghamella echinulata VKM F-439	19.7	Mortierella gemmifera VKM F-1252	15.9
Rhizopus stolonifer CCM F-445	19.4	Mortierella gamsii VKM F-1402	15.3
Rhizopus stolonifer VKM F-399	19.0	Mortierella humilis VKM F-1494	11.3

Strain	GLA (g/L)	Strain	ARA (g/L)
Cunninghamella echinulata VKM F-470	1.2	Mortierella alpina ATCC 32222	1.5
Cunninghamella echinulata VKM F-439	0.9	Mortierella hyalina VKM F-1854	0.7
Cunninghamella echinulata VKM F-531	0.7	Mortierella alpina UBOCC-A-112046	0.7
Absidia glauca CCM 450	0.7	Mortierella gamsii VKM F-1402	0.5
Absidia glauca CCM 451	0.7	Mortierella elongata VKM F-524	0.4
Absidia cylindrospora VKM F-1632	0.6	Mortierella gemmifera VKM F-1631	0.4
Mucor fragilis UBOCC-A-109196	0.6	Mortierella alpina UBOCC-A-112047	0.4
Mucor racemosus UBOCC-A-111127	0.6	Mortierella gemmifera VKM F-1651	0.4
Absidia cylindrospora CCM F-52T	0.5	Mortierella zonata UBOCC-A-101348	0.3
Mucor circinelloides VI 04473	0.5	Mortierella gemmifera VKM F-1252	0.3

Table S2 Total lipid content of biomass (%), total lipid concentration (g/L medium) and calculated cetane number (Ramírez-Verduzco et al.[1]) of the 10 most promising Mucoromycota strains for biodiesel production

Strain	Total lipid (%)	Strain	Total lipid (g/L)	Strain	CN
Absidia glauca CCM 451	47.2	Cunninghamella blakesleeana CCM F-705	8.3	Umbelopsis vinacea CCM F-539	62.8
Absidia glauca CCM 450	39.1	Umbelopsis vinacea CCM F-539	7.9	Umbelopsis vinacea UBOCC-A-101347	62.3
Cunninghamella blakesleeana CCM F-705	36.7	Umbelopsis vinacea UBOCC-A-101347	7.0	Lichtheimia corymbifera CCM 8077	62.1
Absidia coerulea VKM F-627	35.6	Absidia glauca CCM 450	6.5	Rhizopus oryzae CCM 8075	61.5
Absidia glauca UBOCC-A-101330	34.8	Absidia glauca CCM 451	6.2	Cunninghamella blakesleeana CCM F-705	61.1
Umbelopsis vinacea CCM F-539	34.8	Umbelopsis isabellina VKM F-525	6.0	Umbelopsis isabellina UBOCC-A-101351	60.9
Absidia glauca CCM F-444	33.3	Absidia coerulea CCM 8230	5.3	Lichtheimia corymbifera VKM F-513	60.8
Umbelopsis vinacea CCM 8333	31.8	Cunninghamella blakesleeana VKM F-993	5.3	Umbelopsis isabellina UBOCC-A-101350	60.8
Mucor hiemalis UBOCC-A-101359	31.8	Lichtheimia corymbifera CCM 8077	4.8	Cunninghamella blakesleeana VKM F-993	60.7
Mucor hiemalis UBOCC-A-112185	31.5	Absidia glauca CCM F-444	4.6	Umbelopsis vinacea CCM 8333	60.7

Table S3 Tentative peak assignment in FTIR spectra of filamentous fungi. Abbreviations: asym, antisymmetric; sym, symmetric; str, stretching; def, deformation [2-8]

No.	**Frequency (cm^{-1})**	**Assignment**	**Main biomolecules**
1	~3300	N-H str (amide A), O-H str	Protein, Carbohydrate
2	3010	=C-H str	Lipid
3	2955	C-H str (asym) of -CH_3	Lipid
4	2925	str of >CH_2 of acyl chains (asym)	Lipid
5	2850	str of CH_2 of acyl chains (sym)	Lipid
6	1745	C=O str.	Lipid
7	1680-1640	Amide I band (C=O str)	Protein
8	1580-1520	Amide II (CONH bending)	Protein
9	1465	CH_2 def	Lipid
10	1410	Amide III band (C-N str)	Protein
11	1380	CH_3 bending	Lipid
12	1265	P=O str (asym) of >PO_2 phosphodiesters	Polyphosphate, Phospholipid
13	1155	C-O-C stretch	Lipid
14	1080	P O str (sym) of >PO_2	Polyphosphate, Phospholipid
15	900-1200	C-O str, C-C str., C-O-H def. C-O-C def.	Carbohydrate
16	875	P-O-P stretching	Polyphosphate, Phospholipid
17	725	CH_2 def	Lipid

Table S4 Prediction of total lipid content in fungal biomass from (preprocessed) FTIR spectra for all strains and at the genus level (*Amylomyces* and *Lichtheimia* were treated together with *Mucor* and *Absidia*, respectively)

R^2 between GC total lipid and FTIR data	**Peak height [a] 1745 cm^{-1}**	**Peak height [b] 1745 cm^{-1}**	**Peak height ratio[b] 1745/1655 cm^{-1}**	**PLSR [c] (factors/RMSECV)**
All (100)	0.41	0.47	0.22	0.72 (3/4.20)
***Mucor*/*Amylomyces* (43)**	0.36	0.42	0.46	0.80 (6/2.72)
***Rhizopus* (9)**	0.31	0.54	0.50	0.73 (2/1.80)
***Umbelopsis* (9)**	0.04	0.07	0.00	0.43 (8/2.60)
***Absidia*/*Lichtheimia* (13)**	0.44	0.21	0.12	0.62 (9/3.75)
***Cunninghamella* (5)**	0.14	0.17	0.01	0.77 (5/2.27)
***Mortierella* (21)**	0.37	0.66	0.15	0.78 (10/3.45)

a EMSC correction with linear and quadratic component were used in the full spectral range (4000-500 cm^{-1})

b Second derivative spectra were obtained by the Savitzky–Golay (S-G) algorithm using windows size (ws) 15 and a second degree polynomial in the full spectral range (4000-500 cm^{-1}) followed by EMSC

c Spectra were prepocessed with (S-G) algorithm (ws 15, 2nd degree polynomial) followed by EMSC and cross-validation with leave-one-biological-replicate-out method. The maximum amount of PLSR components was 10. RMSECV: Root Mean Square Error of Cross Validation

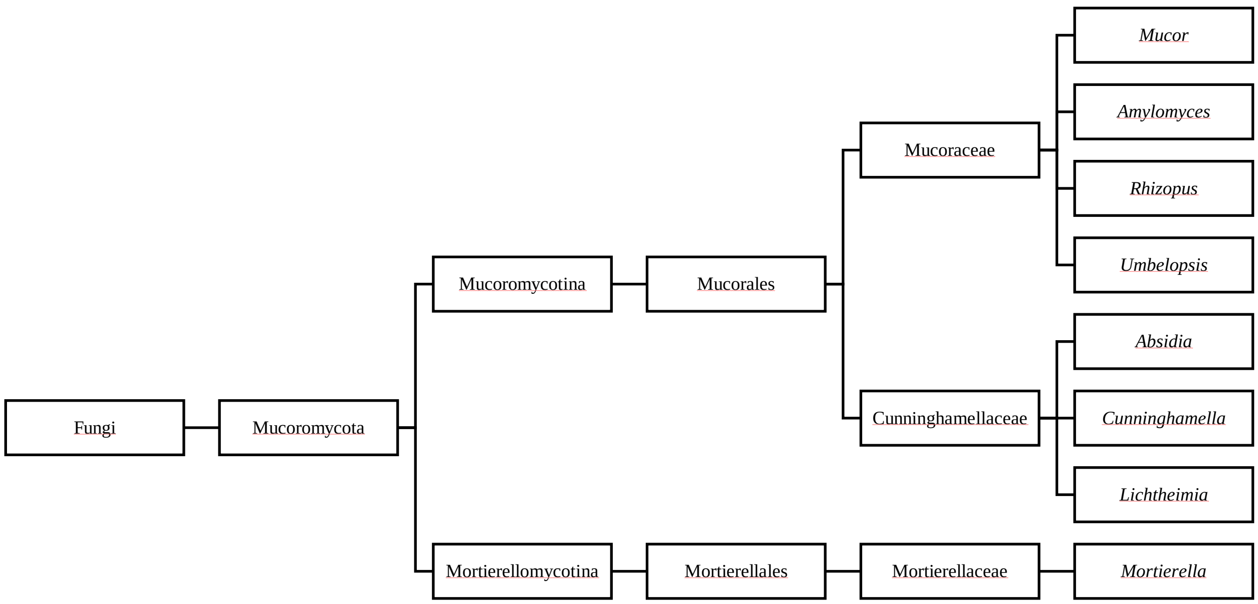

Fig. S1 Classification of Mucoromycota fungi used in the study (according to Westerdijk Fungal Biodiversity Institute [9])

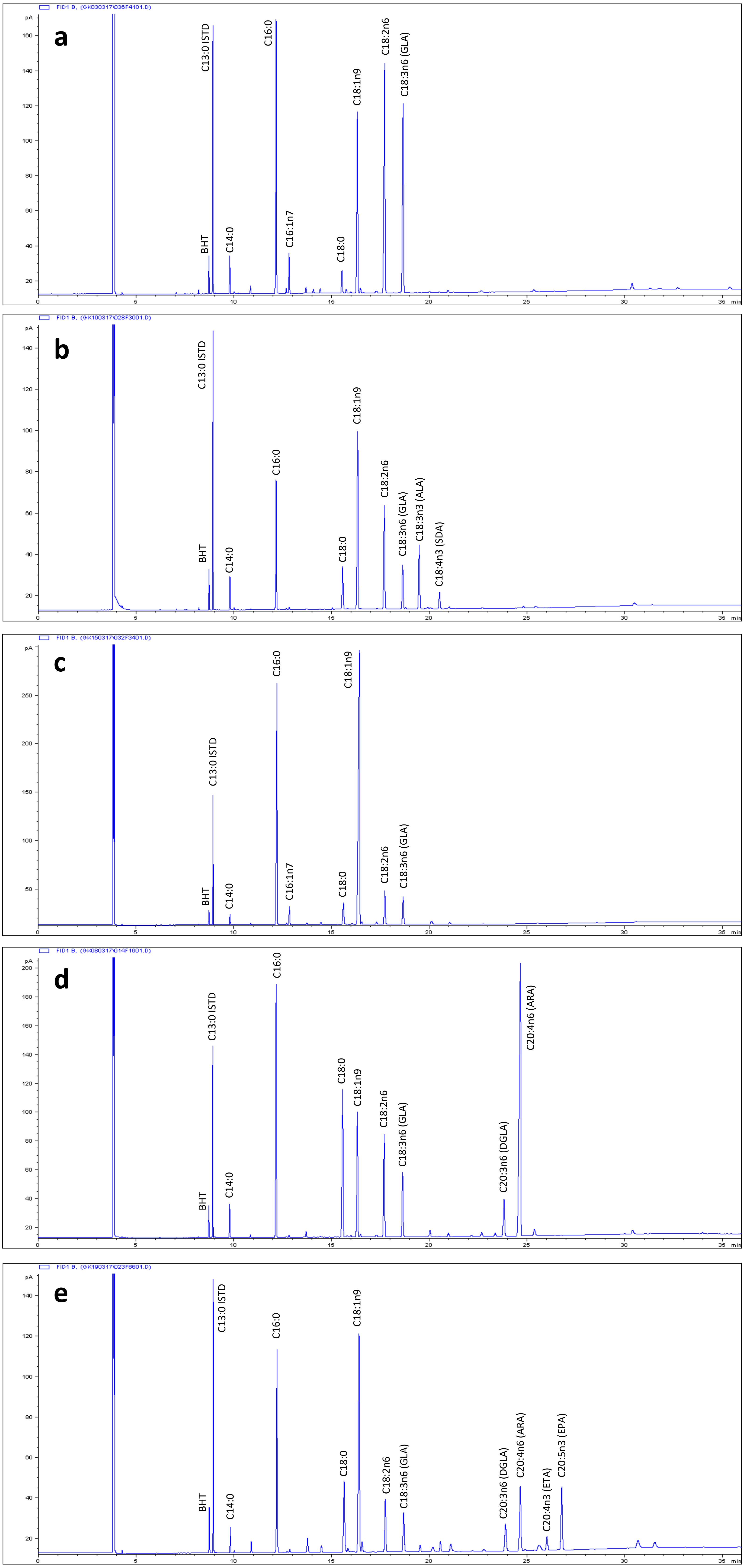

Fig. S2 GC-FID chromatograms of Mucoromycota fungi fatty acid composition (examples) **a)** *Mucor fragilis* UBOCC-A-109196, **b)** *Mucor flavus* CCM 8086 (15°C) **c)** *Umbelopsis vinacea* CCM F-539, **d**) *Mortierella alpina* ATCC 32222, **e)** *Mortierella humilis* VKM-1494 (15°C). BHT: butylated hydroxytoluene (antioxidant)

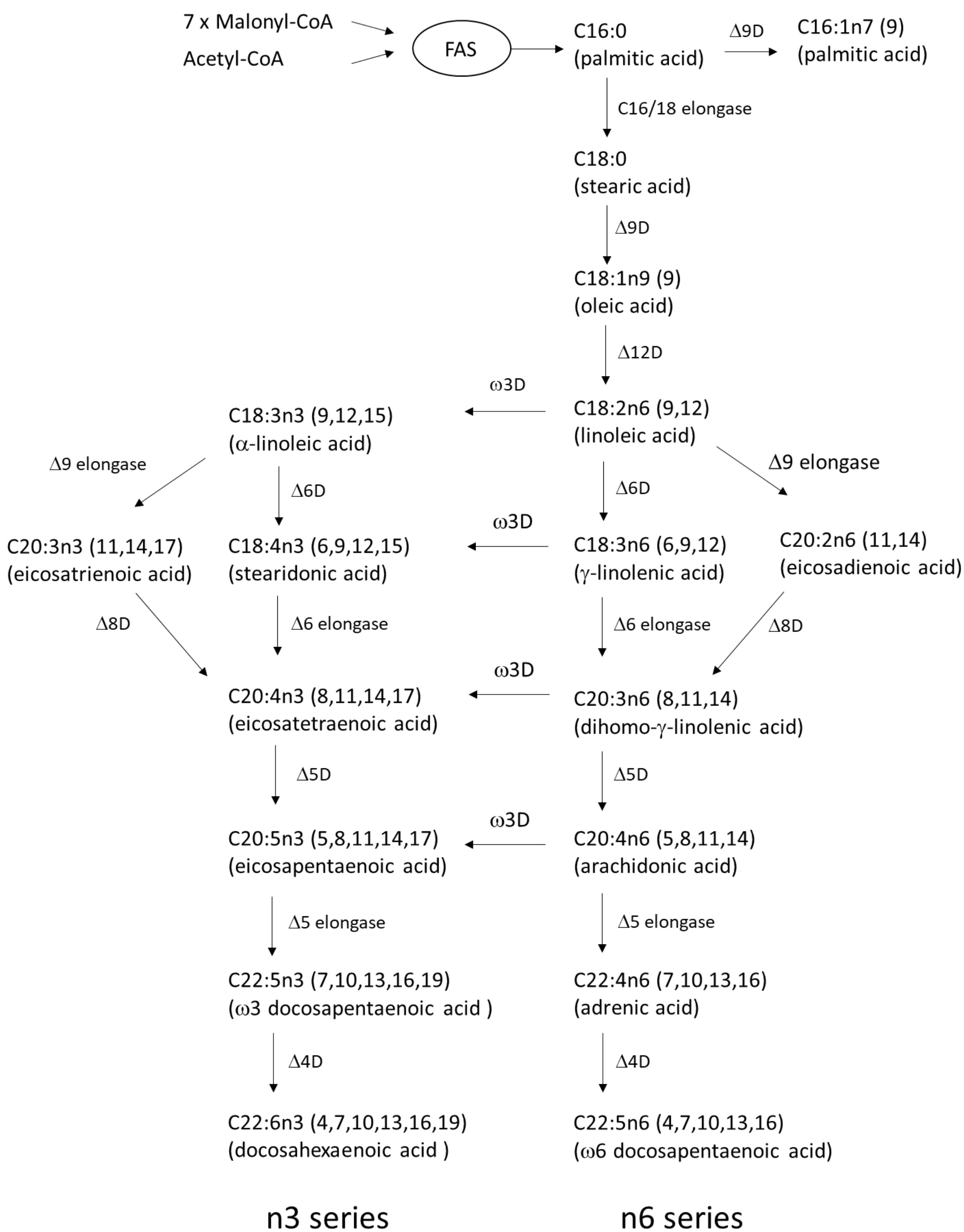

Fig. S3 Pathways for the formation of PUFA in microorganisms. FAS: fatty acid synthase, D: desaturase, Δ: position of double bond from carboxylic end. Adapted from Ratledge, 2004 [10], Lee *et al.*, 2016 [11], and Ruiz-López *et al.*, 2012 [12]

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