SUPPLEMENTARY MATERIAL

Production of Polyunsaturated Fatty Acids and Lipids from Autotrophic, Mixotrophic and Heterotrophic cultivation of *Galdieria* sp. strain USBA-GBX-832

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Table S1. Analysis of variance (ANOVA) to compare the mean values obtained when *Galdieria* sp. USBA-GBX-832 was growing at different temperatures under heterotrophic conditions. SS: Sum of squares, df: Degrees of freedom; *Significance at p <0.05.

Source	Dependent variable	Sum of square	df	Mean square	F	*P* value
Model	**Biomass Productivity**	855505,228[a]	18	47528,068	523,493	,000
	Lipid productivity	832,804[b]	18	46,267	221,491	,000
	%Lipids	825,174[c]	18	45,843	419,791	,000
	%∑ PUFA	50246,217[d]	18	2791,456	3336,328	,000
Temperature	**Biomass Productivity**	97024,080	1	97024,080	1068,662	,000
	Lipid productivity	1,270	1	1,270	6,080	,019
	%Lipids	68,394	1	68,394	626,293	,000
	%∑ PUFA	2568,181	1	2568,181	3069,471	,000
Substrate	**Biomass Productivity**	42398,940	8	5299,867	58,375	,000
	Lipid productivity	62,295	8	7,787	37,278	,000
	%Lipids	40,010	8	5,001	45,797	,000
	%∑ PUFA	189,491	8	23,686	28,310	,000
Temperature * Substrate	**Biomass Productivity**	17157,100	8	2144,637	23,622	,000
	Lipid productivity	46,217	8	5,777	27,656	,000
	%Lipids	36,495	8	4,562	41,774	,000
	%∑ PUFA	268,212	8	33,526	40,071	,000
Error	**Biomass Productivity**	3268,448	36	90,790		
	Lipid productivity	7,520	36	,209		
	%Lipids	3,931	36	,109		
	%∑ PUFA	30,121	36	,837		
Total	**Biomass Productivity**	858773,676	54			
	Lipid productivity	840,324	54			
	%Lipids	829,105	54			
	%∑ PUFA	50276,337	54			

a. R^2 = ,996 (Adj R-Squared = ,994)

b. R^2 = ,991 (Adj R-Squared = ,987)

c. R^2 = ,995 (Adj R-Squared = ,993)

d. R^2 = ,999 (Adj R-Squared = ,999)

Table S2. Experimental design used to evaluate other culture variables that favored higher levels of biomass production and lipid accumulation

Test	pH	Galactose (mM)	Yeast extract ($g.L^{-1}$)	$(NH_4)_2SO_4$ ($g.L^{-1}$)	Temperature (°C)
1	2.5	2.5	0.5	0.13	25
2	2.5	2.5	0	1.3	25
3	2.5	25	0	0.13	25
4	2.5	25	0.5	1.3	25
5	1.5	25	0	1.3	25
6	1.5	25	0.5	0.13	25
7	1.5	2.5	0.5	1.3	25
8	1.5	2.5	0	0.13	25
9	1.5	25	0.5	1.3	25
10	1.5	25	0	0.13	25
11	1.5	2.5	0	1.3	25
12	1.5	2.5	0.5	0.13	25
13	2.5	25	0	1.3	25
14	2.5	25	0.5	0.13	25
15	2.5	2.5	0.5	1.3	25
16	2.5	2.5	0	0.13	25
17	2.5	2.5	0.5	0.13	45
18	2.5	2.5	0	1.3	45
19	2.5	25	0	0.13	45
20	2.5	25	0.5	1.3	45
21	1.5	25	0	1.3	45
22	1.5	25	0.5	0.13	45
23	1.5	2.5	0.5	1.3	45
24	1.5	2.5	0	0.13	45
25	1.5	25	0.5	1.3	45
26	1.5	25	0	0.13	45
27	1.5	2.5	0	1.3	45
28	1.5	2.5	0.5	0.13	45
29	2.5	25	0	1.3	45
30	2.5	25	0.5	0.13	45
31	2.5	2.5	0.5	1.3	45
32	2.5	2.5	0	0.13	45

Table S3a. Analysis of variance (ANOVA) of the multifactorial experimental design over **biomass productivity** of *Galdieria* sp. USBA-GBX-832

Response	**1**	**Biomass productivity**	
Transform:	**Natural Log**	**Constant:**	**0**

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	59.10	31	1.91	576.10	< 0.0001	**SIGNIFICANT**
A-Temperature	10.88	1	10.88	3288.17	< 0.0001	
B-pH	0.12	1	0.12	35.09	< 0.0001	
C-[Gal]	23.83	1	23.83	7201.78	< 0.0001	
D-Y.E	2.93	1	2.93	886.23	< 0.0001	
E-$(NH_4)_2SO_4$	0.32	1	0.32	95.79	< 0.0001	
AB	0.36	1	0.36	109.04	< 0.0001	
AC	1.19	1	1.19	358.59	< 0.0001	
AD	0.02	1	0.02	7.26	0.0111	
AE	0.11	1	0.11	33.76	< 0.0001	
BC	0.43	1	0.43	128.44	< 0.0001	
BD	0.12	1	0.12	37.68	< 0.0001	
BE	0.22	1	0.22	65.07	< 0.0001	
CD	0.85	1	0.85	257.88	< 0.0001	
CE	0.87	1	0.87	264.41	< 0.0001	
DE	0.01	1	0.01	3.95	0.0555	
ABC	0.77	1	0.77	232.44	< 0.0001	
ABD	0.22	1	0.22	67.59	< 0.0001	
ABE	0.00	1	0.00	1.01	0.3225	
ACD	0.62	1	0.62	186.68	< 0.0001	
ACE	1.20	1	1.20	362.70	< 0.0001	
ADE	0.01	1	0.01	4.24	0.0477	
BCD	1.32	1	1.32	399.48	< 0.0001	
BCE	0.59	1	0.59	178.20	< 0.0001	
BDE	0.45	1	0.45	135.28	< 0.0001	
CDE	0.92	1	0.92	277.55	< 0.0001	
ABCD	0.53	1	0.53	160.94	< 0.0001	
ABCE	1.10	1	1.10	332.68	< 0.0001	
ABDE	1.14	1	1.14	345.45	< 0.0001	
ACDE	2.82	1	2.82	853.23	< 0.0001	
BCDE	0.30	1	0.30	90.06	< 0.0001	
ABCDE	4.83	1	4.83	1458.36	< 0.0001	

Std. Dev.	0.057	**R-Squared**	0.99
Mean	3.55	**Adj R-Squared**	0.99
C.V. %	1.62	**Pred R-Squared**	0.99

Table S3b. Analysis of variance (ANOVA) of the multifactorial experimental design used to evaluate various culture factors over **lipid productivity** of *Galdieria* sp. USBA-GBX-832

Response	**1**	**Lipid productivity**				
Transform:	**Natural Log**	**Constant:**	**0**			
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	54.87	31	1.77	71.24	< 0.0001	**SIGNIFICANT**
A-Temperature	13.82	1	13.82	556.11	< 0.0001	
B-pH	0.01	1	0.01	0.47	0.4981	
C-[Gal]	30.11	1	30.11	1211.80	< 0.0001	
D-Y.E	1.67	1	1.67	67.07	< 0.0001	
E-$(NH_4)_2SO_4$	0.52	1	0.52	21.05	< 0.0001	
AB	0.40	1	0.40	16.28	0.0003	
AC	4.46	1	4.46	179.38	< 0.0001	
AD	0.00	1	0.00	0.00	0.9729	
AE	0.06	1	0.06	2.42	0.1294	
BC	0.07	1	0.07	2.69	0.1105	
BD	0.19	1	0.19	7.50	0.0100	
BE	0.23	1	0.23	9.39	0.0044	
CD	0.03	1	0.03	1.09	0.3053	
CE	0.06	1	0.06	2.26	0.1426	
DE	0.00	1	0.00	0.11	0.7410	
ABC	0.25	1	0.25	10.00	0.0034	
ABD	0.11	1	0.11	4.23	0.0479	
ABE	0.08	1	0.08	3.06	0.0899	
ACD	0.09	1	0.09	3.75	0.0618	
ACE	0.01	1	0.01	0.22	0.6389	
ADE	0.03	1	0.03	1.12	0.2982	
BCD	0.65	1	0.65	26.14	< 0.0001	
BCE	0.04	1	0.04	1.57	0.2199	
BDE	0.02	1	0.02	0.95	0.3368	
CDE	0.20	1	0.20	8.07	0.0078	
ABCD	0.07	1	0.07	2.84	0.1019	
ABCE	0.07	1	0.07	2.80	0.1041	
ABDE	0.05	1	0.05	1.99	0.1681	
ACDE	0.51	1	0.51	20.46	< 0.0001	
BCDE	0.25	1	0.25	10.25	0.0031	
ABCDE	0.83	1	0.83	33.36	< 0.0001	

Std. Dev.	0.16	**R-Squared**	0.99
Mean	0.61	**Adj R-Squared**	0.97

C.V. %	25.77	**Pred R-Squared**	0.94

Table S3c. Analysis of variance (ANOVA) of multifactorial experimental design used to evaluate various culture factors over **% PUFA** of *Galdieria* sp. USBA-GBX-832

Response	3	**%PUFA**	
Transform:	Natural Log	Constant:	0

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	20.27	31	0.65	67.00	< 0.0001	**SIGNIFICANT**
A-Temperature	0.51	1	0.51	52.56	< 0.0001	
B-pH	3.55	1	3.55	364.13	< 0.0001	
C-[Gal]	0.23	1	0.23	23.88	< 0.0001	
D-Y.E	0.13	1	0.13	13.27	0.0009	
E-$(NH_4)_2SO_4$	1.03	1	1.03	105.40	< 0.0001	
AB	0.06	1	0.06	5.75	0.0225	
AC	1.84	1	1.84	188.98	< 0.0001	
AD	0.53	1	0.53	54.70	< 0.0001	
AE	0.09	1	0.09	8.96	0.0053	
BC	0.02	1	0.02	2.55	0.1203	
BD	0.04	1	0.04	4.40	0.0440	
BE	1.14	1	1.14	117.11	< 0.0001	
CD	0.86	1	0.86	88.02	< 0.0001	
CE	0.07	1	0.07	7.44	0.0103	
DE	0.01	1	0.01	0.58	0.4523	
ABC	1.70	1	1.70	174.22	< 0.0001	
ABD	0.45	1	0.45	46.19	< 0.0001	
ABE	0.79	1	0.79	81.43	< 0.0001	
ACD	0.74	1	0.74	75.71	< 0.0001	
ACE	0.53	1	0.53	53.92	< 0.0001	
ADE	1.54	1	1.54	157.47	< 0.0001	
BCD	0.80	1	0.80	81.97	< 0.0001	
BCE	0.12	1	0.12	12.45	0.0013	
BDE	0.00	1	0.00	0.41	0.5284	
CDE	1.27	1	1.27	130.03	< 0.0001	
ABCD	0.12	1	0.12	11.87	0.0016	
ABCE	0.59	1	0.59	59.97	< 0.0001	
ABDE	0.72	1	0.72	73.72	< 0.0001	
ACDE	0.05	1	0.05	4.87	0.0346	
BCDE	0.72	1	0.72	73.81	< 0.0001	
ABCDE	0.01	1	0.01	1.30	0.2632	

Std. Dev.	0.10	**R-Squared**	0.98
Mean	3.26	**Adj R-Squared**	0.97

C.V. %	3.03	**Pred R-Squared**	0.94

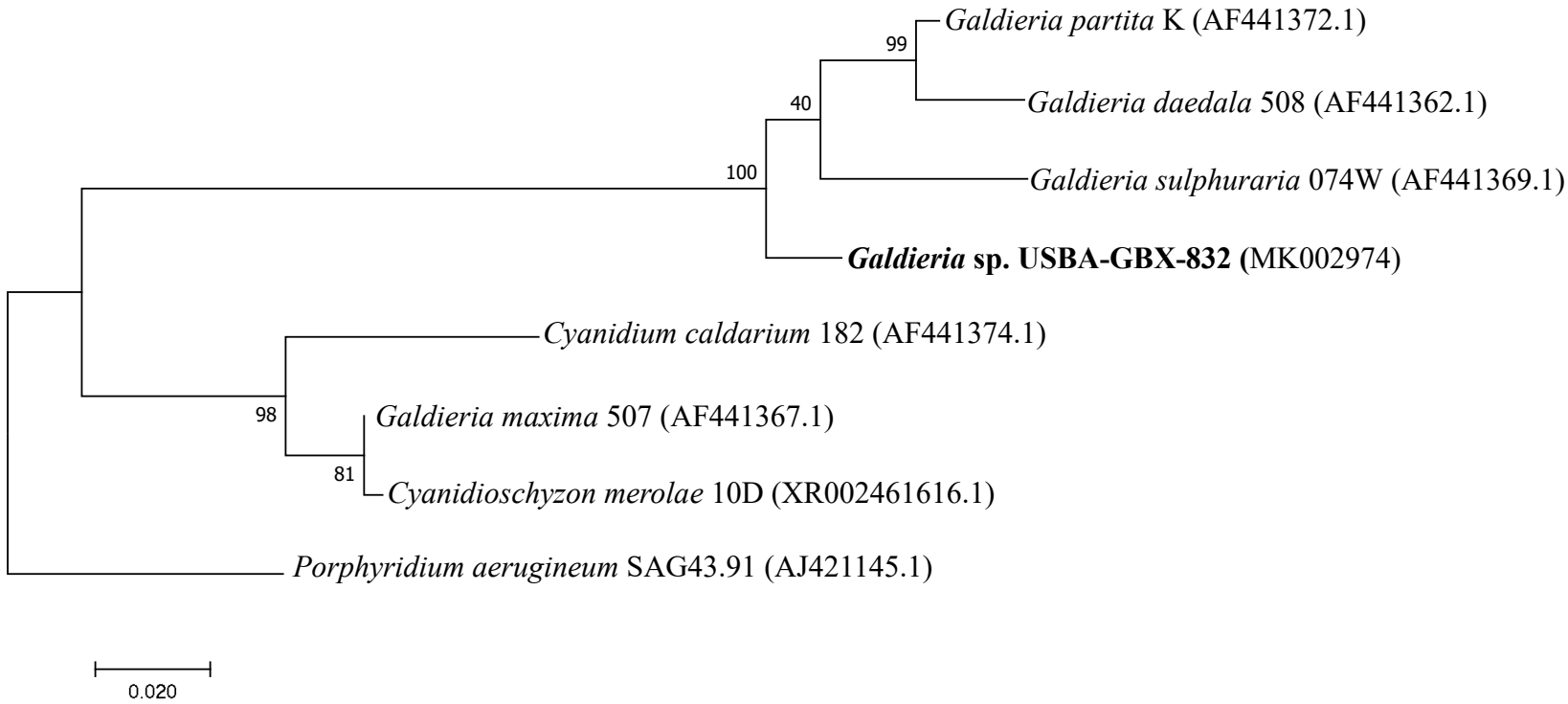

Figure S1. Phylogenetic tree of *Galdieria* sp. USBA-GBX-832 inferred from alignment of 18S rRNA gene and recognized type species of Family Cyanidiaceae. The tree was reconstructed by using the Maximum Likelihood method based on the Tamura-Nei model. Numbers represent percentages from 1000 replicate bootstrap sampling. Bar, 0.02 substitutions per nucleotide position. The microalgae *Porphyridium aerugineum* SAG43.91 (AJ421145.1) was used as outgroup.

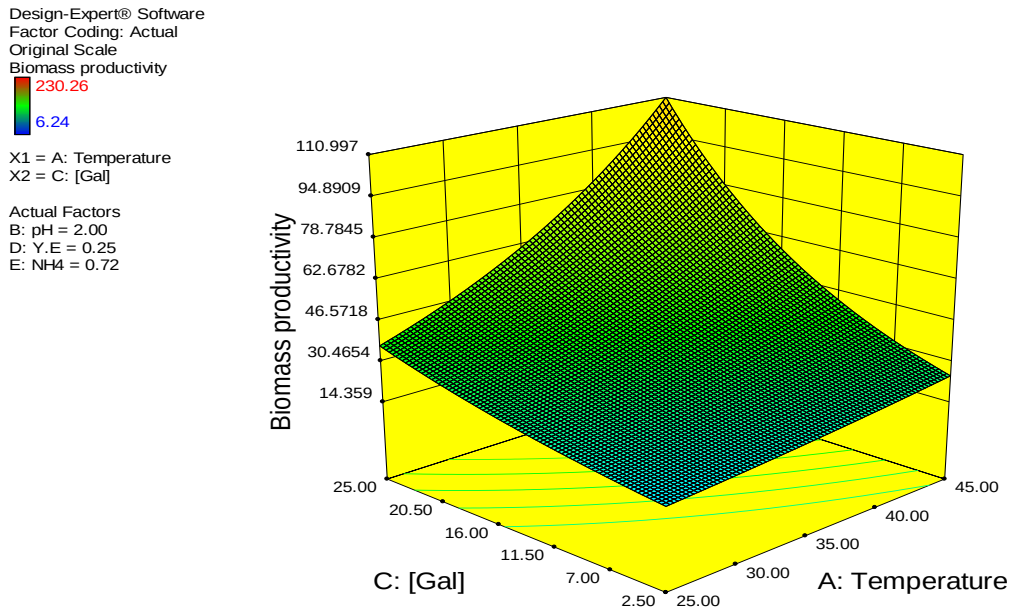

Figure S2a. Response surface curve showing the effect of carbon concentration and temperature over biomass productivity of *Galdieria* sp. USBA-GBX-832

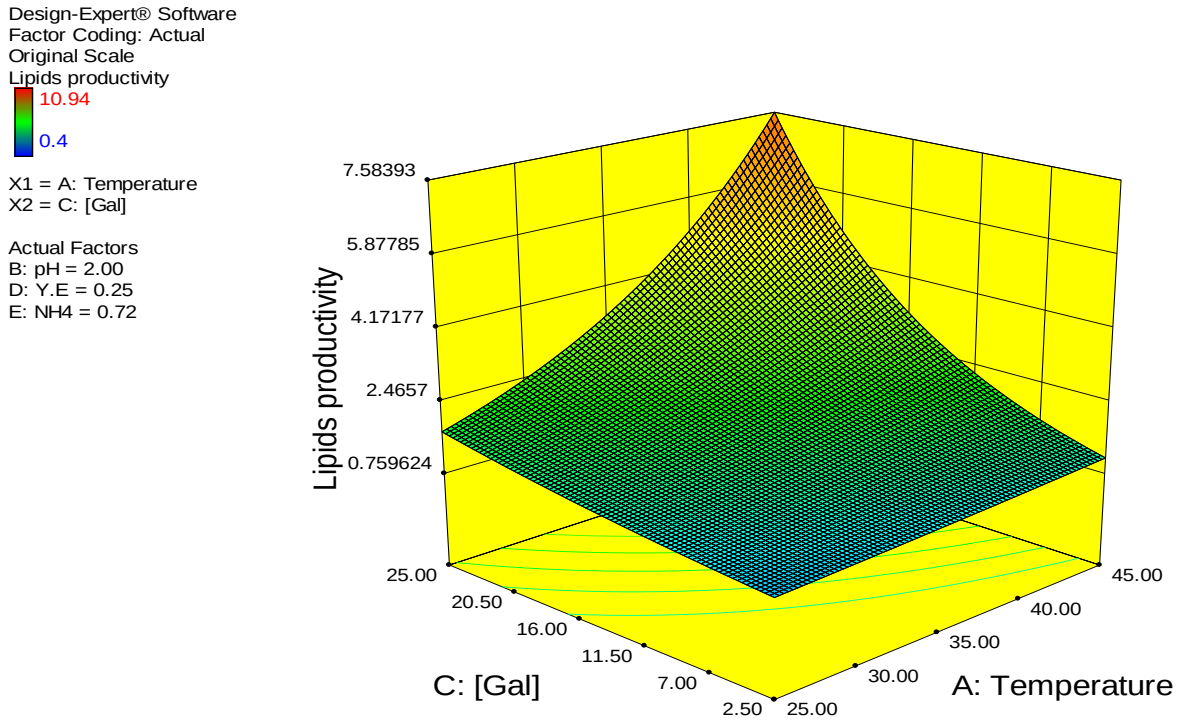

Figure S2b. Response surface curve showing the effect of carbon concentration and temperature over total lipid productivity of *Galdieria* sp. USBA-GBX-832

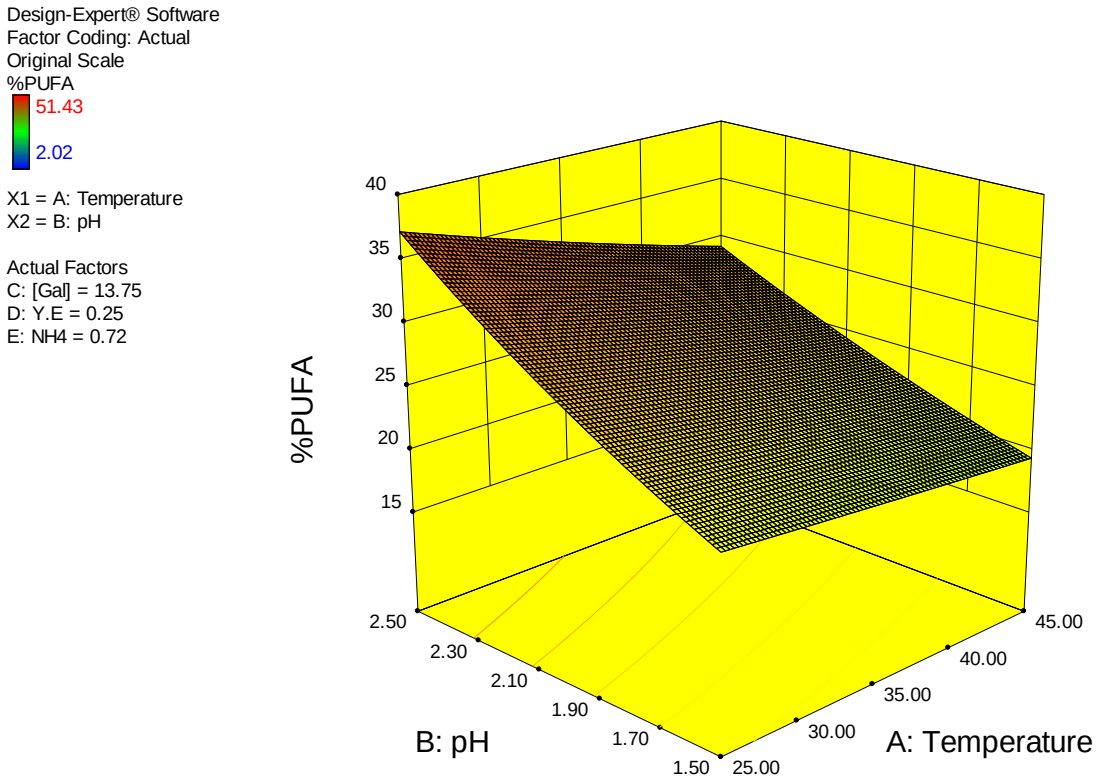

Figure S2c. Response surface curve showing the effect of pH and temperature over percentage of polyunsaturated fatty acid productivity (PUFA) of *Galdieria* sp. USBA-GBX-832

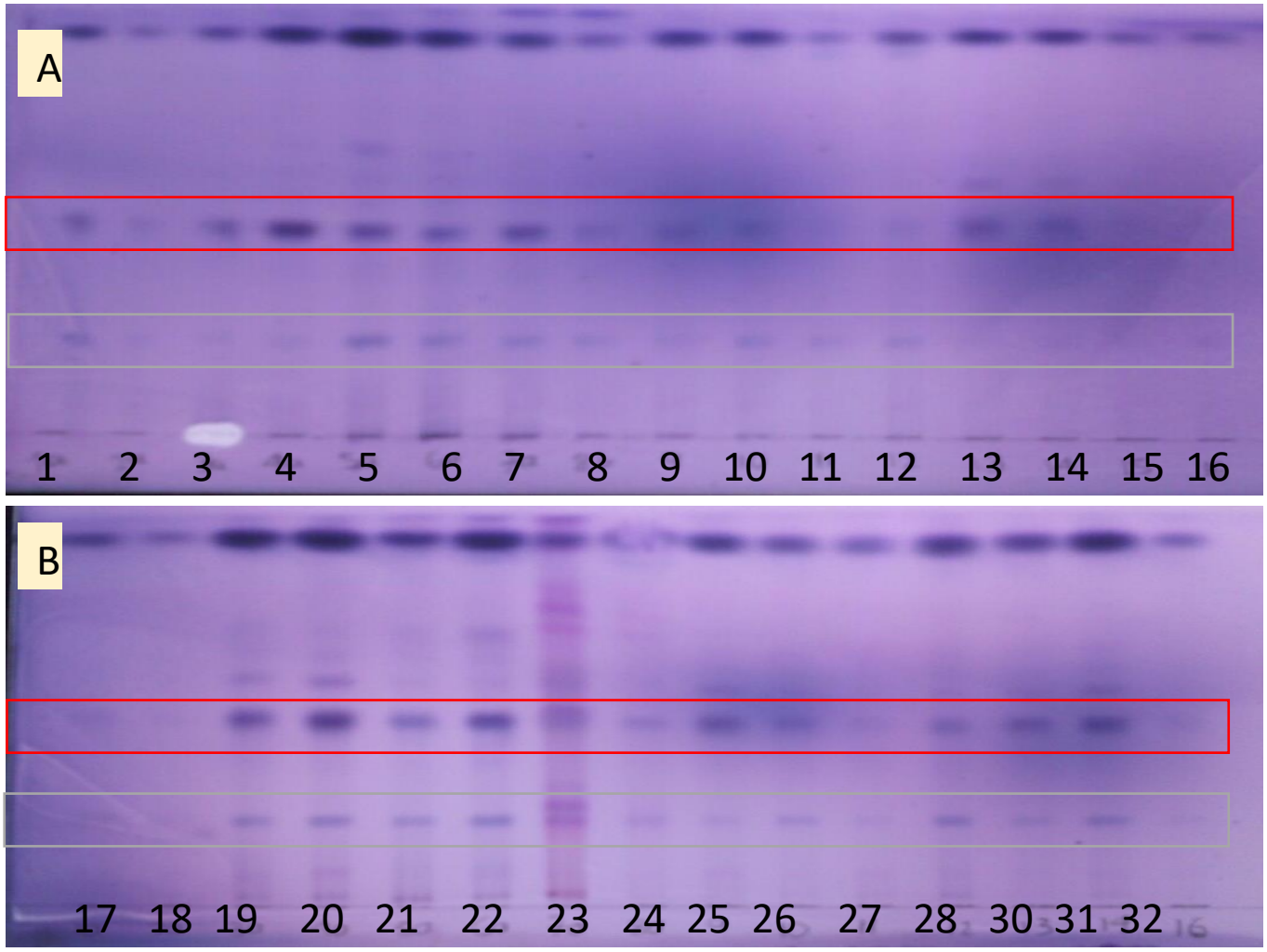

Figure S3. Evaluation of neutral lipids through TLC plates. A) treatments at 45 ° C and B) treatments at 25 ° C. Solvents hexane: ethyl acetate (7: 3). Revealed with *p*-anisaldehyde. The red boxes show the position of triacylglycerides ($C_{16:0}$) and the gray boxes the position of free fatty acids.