



Bioreactor Co-Cultivation of High Lipid and Carotenoid Producing Yeast *Rhodotorula kratochvilovae* and Several Microalgae under Stress

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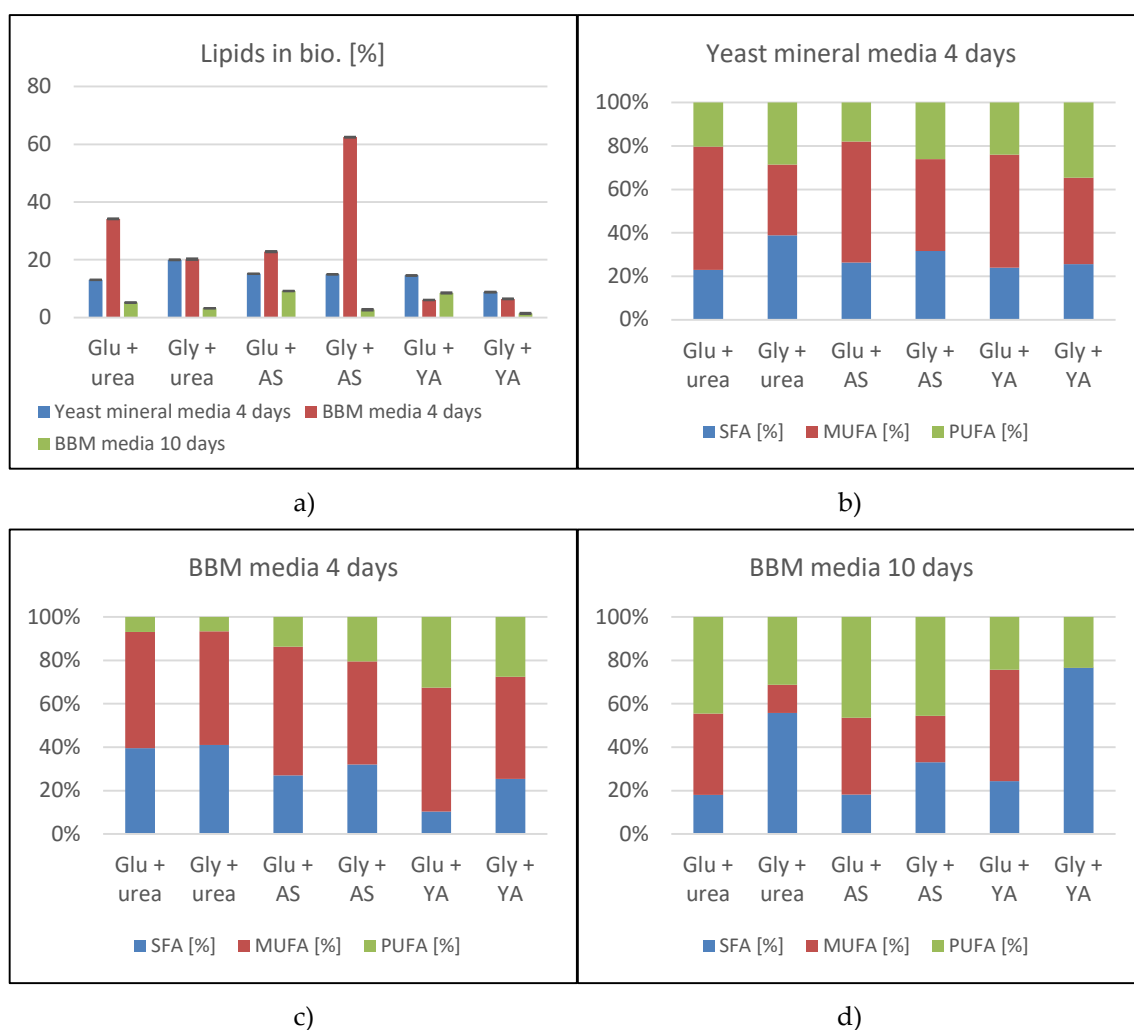


Figure S1. *Rhodotorula kratochvilovae* growth on different media types: lipid production and fatty acid profile GC analysis

a) - Total lipid production [%] b) - FA profile on yeast mineral media 4-day cultivation; c) - FA profile on BBM media 4 days cultivation; d) - FA profile on BBM media 10 days cultivation.

Abbreviations: Glu – Glucose, Gly – Glycerol, AS – Ammonium sulphate, YA – Yeast autolysate

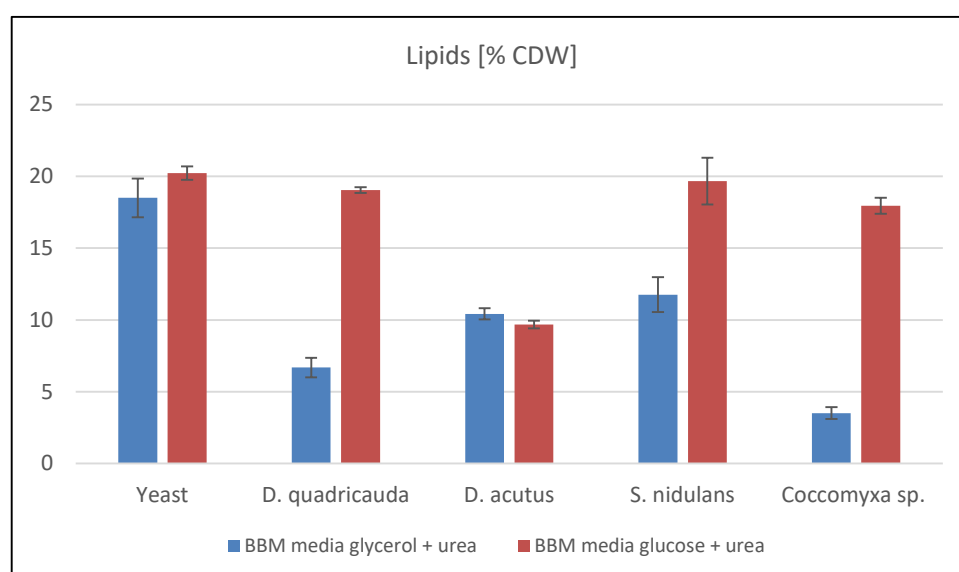


Figure S2. *R. kratochvilovae* lipid production in small-scale co-cultivation experiments

Abbreviations: Yeast – *Rhodotorula kratochvilovae* control cultivation; D. quadricauda – Co-cultivation with *Desmodesmus quadricauda*; D. acutus – Co-cultivation with *Desmodesmus acutus*; S. nidulans– Co-cultivation with *Synechococcus nidulans*; Coccomyxa sp. – Co-cultivation with *Coccomyxa* sp.

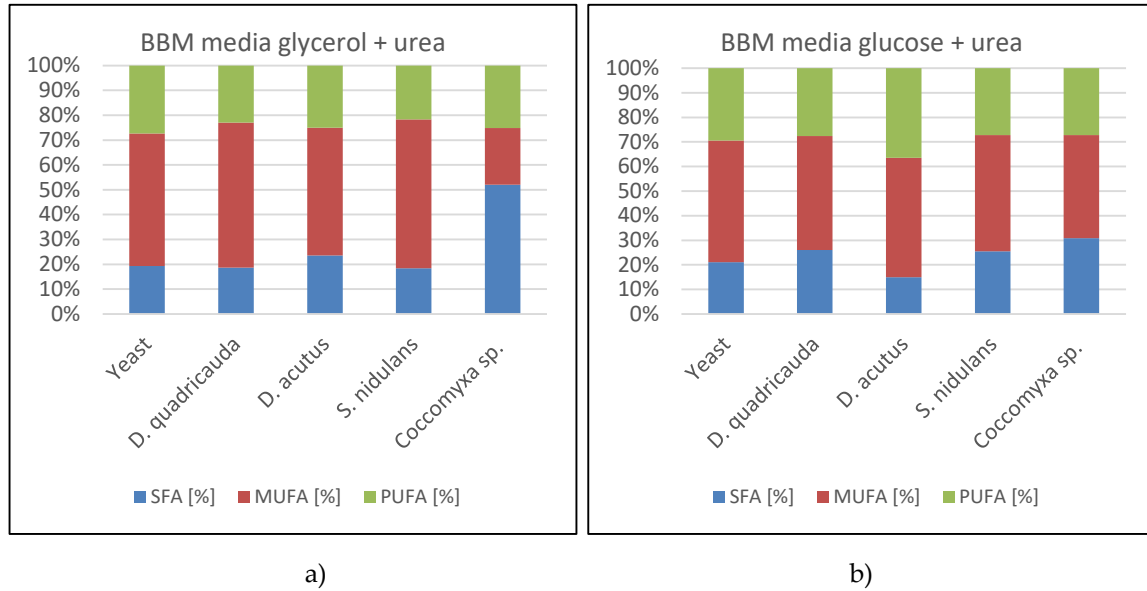


Figure S3. *R. kratochvilovae* fatty acid profile in small-scale co-cultivation experiments

a) - FA profile analysis of *Rhodotorula kratochvilovae* co-cultivation on BBM media with glycerol and urea; b) - FA profile analysis of *Rhodotorula kratochvilovae* co-cultivation on BBM media with glucose and urea

Abbreviations: Yeast – *Rhodotorula kratochvilovae* control cultivation; D. quadricauda – Co-cultivation with *Desmodesmus quadricauda*; D. acutus – Co-cultivation with *Desmodesmus acutus*; S. nidulans– Co-cultivation with *Synechococcus nidulans*; Coccomyxa sp. – Co-cultivation with *Coccomyxa* sp.

Table S1. 2nd Phase of co-cultivation experimental scheme

BBM mineral media							
Carbon source	Glucose	Glucose	Glucose	Glycerol	Glycerol	Glycerol	-
Nitrogen source	Urea	(NH ₄) ₂ SO ₄	NaNO ₃	Urea	(NH ₄) ₂ SO ₄	NaNO ₃	NaNO ₃

Table S2. Experimental scheme of 4th phase of co-cultivation

Yeast	Yeast: Algae 1:1	Yeast: Algae 1:2	Yeast: Algae 1:4	Algae	Algae
BBM+ C	BBM+ C	BBM+ C	BBM+ C	BBM+ C	BBM

Table S3. Bioreactor process values during co-cultivation

Media volume	2.0 L
Stirring	300 – 800 rpm – regulated by oxygen consumption
pH	6.5
pO ₂	30%
Temperature	22 °C

Aeration	2 L per minute
Illumination	300 ol·m ² ·s ⁻¹ of photons

Table S4. HPLC gradient analysis

Time [min]	Mobile phase A	Mobile phase B
0.0	100 %	0 %
13.0	0 %	100 %
19.0	0 %	100 %
20.0	100 %	0 %
25.0	100 %	0 %

Table S5. Temperature programme of GC analysis

	<i>Retention time</i> (min)	<i>gradient</i> (°C·min ⁻¹)	<i>temperature</i> (°C)	<i>retention</i> (min)
1	0.000	start	-	-
2	1.000	0.0	80.0	1.0
3	5.000	15.0	140.0	0.0
4	21.667	3.0	190.0	0.0
5	25.467	25.0	260.0	1.0
6	25.467	stop	-	-

Table S6. HPLC analysis of *R. kratochvilovae*: 4-day cultivation on standard yeast media (Part A) and 10-day cultivation on BBM media (Part B). Concentrations of metabolites are listed in mg/g of dry biomass.

Part A 4-day cultivation on yeast media						
	Betacarotene	Torulene	Torularhodin	Total carotenoids	Ergosterol	Coenzyme Q
Glu + urea	2.044±0.082	0.161±0.065	0.649±0.057	4.929±0.067	3.198±0.101	1.764±0.103
Gly + urea	3.626±0.078	0.140±0.059	0.885±0.082	6.637±0.088	4.485±0.127	0.066±0.014
Gly + AS	2.649±0.071	0.439±0.064	0.688±0.091	7.806±0.097	6.024±0.098	0.786±0.106
Glu + AS	1.880±0.058	0.083±0.071	0.518±0.087	5.356±0.048	5.091±0.108	1.166±0.099
Gly +YA	1.886±0.083	0.114±0.058	0.379±0.085	6.366±0.071	3.941±0.078	1.262±0.120
Glu +YA	1.417±0.078	-	0.033±0.072	3.055±0.083	2.350±0.106	2.041±0.089

Part B 10-day cultivation on BBM media						
	Betacarotene	Torulene	Torularhodin	Total carotenoids	Ergosterol	Coenzyme Q
Glu + urea	0.434±0.102	0.014±0.028	0.088±0.078	0.963±0.097	0.922±0.070	0.241±0.043
Gly + urea	0.841±0.097	0.161±0.034	0.345±0.064	3.011±0.142	4.017±0.103	0.937±0.081
Gly + AS	0.425±0.087	0.115±0.037	0.347±0.047	2.123±0.131	1.804±0.074	0.939±0.078
Glu + AS	0.175±0.039	-	0.041±0.032	0.455±0.087	1.624±0.099	0.396±0.068
Gly +YA	0.574±0.114	-	0.181±0.049	1.425±0.076	1.810±0.086	0.180±0.047
Glu + YA	0.709±0.106	0.066±0.047	0.210±0.037	1.809±0.088	2.116±0.047	0.347±0.065

Abbreviations: Glu – Glucose, Gly – Glycerol, AS – Ammonium sulphate, YA – Yeast autolysate

Table S7. *R. kratochvilovae* small-scale 10-day co-cultivation on BBM media (glucose + urea).

Strain	Betacarotene	Lutein	Total carotenoids	chlorophyll A	Chlorophyll B	Coenzyme Q	Ergosterol
<i>S. nidulans</i>	0.276±0.032	0.002±0.001	1.096±0.163	0.052±0.009	0.810±0.048	1.424±0.103	2.198±0.135
<i>D. quadricauda</i>	0.798±0.103	0.014±0.006	2.191±0.207	0.092±0.007	0.011±0.002	0.980±0.046	1.662±0.086
<i>Coccomyxa</i> sp.	0.209±0.025	0.004±0.002	0.645±0.103	0.020±0.005	0.018±0.006	0.820±0.060	1.444±0.204
<i>D. acutus</i>	0.850±0.097	0.008±0.003	2.133±0.182	0.140±0.032	0.043±0.018	2.417±0.146	3.067±0.148
<i>R. kratochvilovae</i>	0.702±0.121	0	1.827±0.065	0	0	1.248±0.097	1.585±0.115

Note: Concentrations of metabolites are listed in mg/g biomass.