Media screening for obtaining *Haematococcus pluvialis* red motile macrozooids rich in astaxanthin and fatty acids

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Supplementary information

Table S1 Sources of astaxanthin from natural origins

Species	**Class**	**Astaxanthin content (% DW)**	**Reference**
Haematococcus pluvialis	Chlorophyceae	4.0	[2]
Chlorella zofingiensis	Chlorophyceae	0.7	[77]
Scenedesmus vacuolatus	Chlorophyceae	0.3	[77]
Scotiellopsis oocystiformis	Chlorophyceae	1.1	[77]
Neochloris wimmeri	Chlorophyceae	1.9	[77]
Protosiphon botryoides	Chlorophyceae	1.4	[77]
Chlorococcum sp.	Chlorophyceae	0.2	[78]
Adonis annua	Dicotyledons	1	[79]
Ulva intestinalis	Ulvophyceae	0.02	[80]
Ulva lactuca	Ulvophyceae	0.01	[80]
Catenella repens	Florideophyceae	0.02	[80]
Agrobacterium aurantiacum	Alphaproteobacteria	0.01	[81]
Paracoccus carotinifaciens	Alphaproteobacteria	2.2	[82]
Phaffia rhodozyma/ Xanthophyllomyces dendrorhous	Tremellomycetes	0.01	[83]

Table S2 *H. pluvialis* biomass and astaxanthin productivity

Strain no.	Conditions	Culture time (days)	Biomass yields green stage (g L^{-1} DW)	Biomass productivity green stage (g L^{-1} d^{-1} DW)	Astaxanthin content red stage (mg g^{-1} DW)	Astaxanthin yield red stage (mg L^{-1} DW)	Astaxanthin productivity green + red stages (mg L^{-1} d^{-1} DW)	Ref.
CCAP 34/7	30 L air-lift bioreactor (autotrophic)	90	1.6	0.02	27	43.2	0.5	[27]
UTEX 16	3.7 L Fed-batch bioreactor (mixotrophic)		2.74	0.14	43	64.4	3.2	[84]
NIES-144	2.3 L Maxblend fermentor. pH stat fed-batch (sequential heterotrophic-autotrophic)	30 (20+10)	7	0.35	98	114	3.8	[85]
CCAP 34/8	50 L tubular bioreactor, 0.6 mM nitrate for red stage, continuous one-stage production process outdoors (autotrophic)		0.7	0.6-0.7	6-13	11	3.5-8	[56]
Unknown strain from Aquasearch	Green stage in Aquasearch growth modules (AGMs), followed by open pond astaxanthin induction (autotrophic)	19 (14+5)	0.2-0.36	0.036-0.052	28-30		2.2	[22]
CCAP 34/8	2 L jacked bubble column bioreactor, 2.7 mM nitrate deprivation in red stage, one-stage continuous process indoors (autotrophic)		1.03	0.93	6	6.2	5.6	[43]
NIES-144	Astaxanthin induction using nitrate depletion, with 5 % CO_2 (autotrophic)	28	2.27	0.08	77.2	175.7	6.3	[86]

Strain no.	Conditions	Culture time (days)	Biomass yields green stage (g L^{-1} DW)	Biomass productivity green stage (g L^{-1} d^{-1} DW)	Astaxanthin content red stage (mg g^{-1} DW)	Astaxanthin yield red stage (mg L^{-1} DW)	Astaxanthin productivity green + red stages (mg L^{-1} d^{-1} DW)	Ref.
CCAP 34/8	2L jacketed bubble column bioreactors, one-stage continuous process indoors (autotrophic)			1.9	11		20.8	[42]*
CCAP 34/8	55 L tubular bioreactor, nitrate reduced to < 5 mM for astaxanthin accumulation (autotrophic)	16	7	0.55	20	72	4.5	[87]
SAG 34/1b	1 L cylindrical air-lift double-region bioreactor modified into cone shape (inner core green stage, outer core red stage), batch production (autotrophic)	22 (12+10)	4 (inner core) and 7 (outer core) after day 12 FW**	0.33 FW	49.5	357	16.2	[58]
SCCAP k-0084	500 mL glass columns, two-stage strategy indoors	10 (4+6)	2	0.5	40	115	11.5	[2]
	500 L flat vertical plate bioreactor (green stage), 2000 L tubular bioreactor (red stage), two stage strategy outdoors	10 (4+6)	1.48	0.37	40	101	10.1	
NIES-144	Bubble column fed-batch process (autotrophic)	55 (12.5+ 42.5)	6.7	0.2	36	390	7.2	[88]
NIES-144	1 L flat panel bioreactor with stepwise increase in irradiance, astaxanthin induction using 5 % CO_2 and high light (autotrophic)	13.5 (4.5+9)	1.47	0.33	47	190	14.1	[89]

Strain no.	Conditions	Culture time (days)	Biomass yields green stage (g L^{-1} DW)	Biomass productivity green stage (g L^{-1} d^{-1} DW)	Astaxanthin content red stage (mg g^{-1} DW)	Astaxanthin yield red stage (mg L^{-1} DW)	Astaxanthin productivity green + red stages (mg L^{-1} d^{-1} DW)	Ref.
WBG 26	Double-layer circular pond, two-step one-stage process (autotrophic)	12	1.83	0.15	27.9	51.1	4.3	[62]
NIES-144	3 L fermentor with stepwise increase in irradiance in fed-batch and perfusion processes (mixotrophic)							[90]
	Batch	26 (6+20)	0.96	0.16	49	213	8.2	
	Fed-batch	30 (10+20)	2.8	0.28		437	15.1	
	Perfusion	38 (14+24)	4.48	0.32		602	15.8	
SKLBE ZY-18	Sequential Heterotrophy-Dilution-Photoinduction (SHDP)	27 (17 + 10)	26	1.54	46	64	6.4	[91]

*The publication by Del Río et al. [42] reported the highest astaxanthin productivity (mg $L^{-1}d^{-1}$) to date using the one-stage continuous production process

**FW = Fresh weight

Table S3 Chemical composition of autotrophic and mixotrophic media used in the initial medium preference study for the green stage

Medium component	3N-BBM+V	3N-BBM+V+ SA	BG-11	EG:JM
$NaNO_3$ (mgL^{-1})	750	750	1500	40
Na_2HPO_4 (mgL^{-1})				
K_2HPO_4 (mgL^{-1})	75	75	40	
KH_2PO_4 (mgL^{-1})	175	175		6.2
Na_2HPO_4 $12H_2O$ (mgL^{-1})				18
$MgSO_4$ $7H_2O$ (mgL^{-1})	75	75	75	25
$C_6H_8O_7$ (mgL^{-1})			6	
Ammonium ferric citrate green (mgL^{-1})			6	
$Ca(NO_3)_2$ $4H_2O$ (mgL^{-1})				10
$CaCl_2$ $2H_2O$ (mgL^{-1})	25	25	36	
$CaCl_2$ (mgL^{-1})				5

$FeCl_2\ 6H_2O$ (mgL^{-1})	0.582	0.582		
$CoCl_2\ 6H_2O$ (mgL^{-1})	0.012	0.012		
$ZnCl_2$ (mgL^{-1})	0.03	0.03		
$Co(NO_3)_2\ 6H_2O$ (mgL^{-1})			0.05	
$CuSO_4\ 5H_2O$ (mgL^{-1})			0.08	
Cr_2O_3 (mgL^{-1})				
$MnCl_2\ 4H_2O$ (mgL^{-1})	0.246	0.246	1.81	0.695
Na_2MoO_4 (mgL^{-1})				
$Na_2MoO_4\ 2H_2O$ (mgL^{-1})	0.024	0.024	0.39	
$(NH_4)_6Mo_7)_{24}\ 4H_2O$ (mgL^{-1})				0.5
H_3BO_3 (mgL^{-1})			2.86	1.24
$ZnSO_4\ 7H_2O$ (mgL^{-1})			0.22	
Na_2CO_3 (mgL^{-1})			20	
Na_2-EDTA (mgL^{-1})			1	1.125
FeNa-EDTA (mgL^{-1})				1.125
$C_2H_3NaO_2$ (mgL^{-1})		820		240
Lab-Lemco powder (mgL^{-1})				500
Tryptone (mgL^{-1})				1000
Yeast extract (mgL^{-1})				1000
NaCl (mgL^{-1})	25	25		
$NaHCO_3$ (mgL^{-1})				7.95
Thiamine-HCl (mgL^{-1})	0.012	0.012		0.02
B_{12} (mgL^{-1})	0.01	0.01		0.02

Table S4 Composition of FM:FB. Nutrient concentrations (guaranteed minimum concentrations) in mg L^{-1} calculated from information from GHE, France

Constituent	mgL^{-1}
Nitrogen as ammonium	10
Nitrogen as nitrate	40
Phosphate (P_2O_5)	250
Potassium (K_2O)	200
Magnesium	250
Sulphur	50
Boron	0.1
Calcium	70
Copper	0.1
Iron	1.2
Manganese	0.4
Molybdenum	0.04
Zinc	0.15

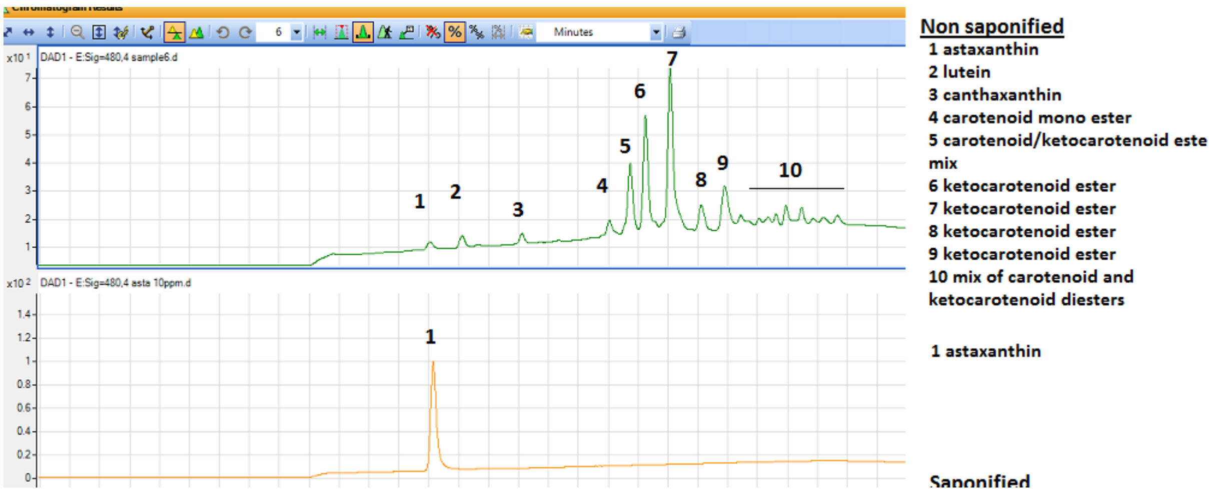

Figure S1 Identification of carotenoid peaks by Liquid Chromatography-Mass Spectrometry (LC-MS)

Table S5 Liquid Chromatography Mass Spectrometry (LC-MS) data

Peak No	*m/z* [M+H]	Putative Identity
1	619	Astaxanthin[a]
2	591	Lutein[a]
3	**565**, 588 (+Na)	Canthaxanthin[a]
4	864.7	Unknown Astaxanthin monoester[b]
5	864.7	Unknown Astaxanthin monoester[b]
6	859.6	Astaxanthin monoester (18:2)
6a	866.7	+ 2 amu >than peaks 4 & 5
7	**861.6**, 883.6 (+Na)	Astaxanthin monoester 18:1
8	898.9	Unknown Astaxanthin ester
9	868.7	Unknown Astaxanthin ester
10	870.7, 872.7, 874.8, 876.8	Mix of esterified forms

Values in bold are major signals.

a – identity confirmed by standards

b – possibly astaxanthin monoester (14:0 + NH_3) using data from Murphy et al. [92]

Most IDs are from Holtin et al. [49] and Frassanito et al. [93]

The main peaks 6 & 7 actually match with the relative abundance shown in this paper. The more hydrophobic peaks after peak 9 are probably diesters which do not yield detectable M+H ions, possibly due to multiply charged forms.

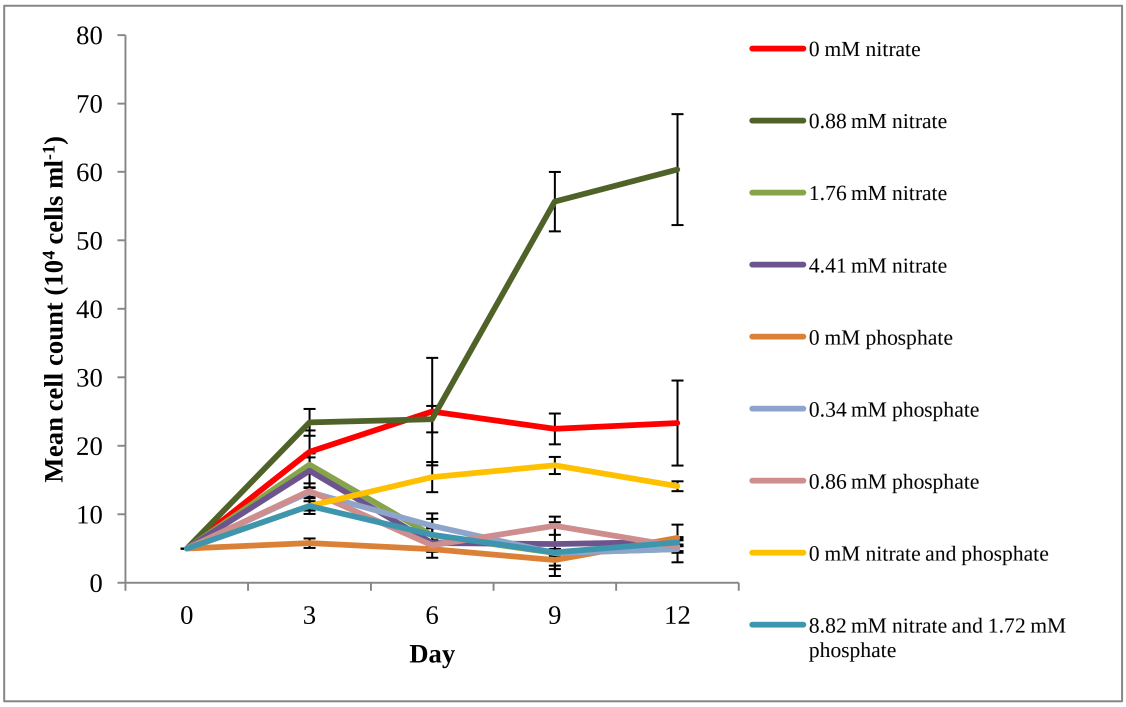

Figure S2 Effect of nitrate and phosphate concentration on growth in *H. pluvialis* (n = 3)

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