fermentation

Supplementary Materials

1. Supplementary method

Lipid extraction

Cells in 25 mL cultures were harvested by centrifugation (4,470× g) at 4 °C for 5 min. Cell pellets were transferred in 2 mL screw-capped microcentrifuge tubes with ~250 µL 425 - 600µm acid-washed glass beads (Sigma) and 1 mL methanol, 10 µL 10 mg heptadecanoic acid mL^{-1} (Alfa Aesar) was added as internal standard. The cells were lysed using the Disruptor Genie Cell Disruptor (Scientific Industries) twice for 6 minutes each time. The lysed cells (in 1 mL methanol) was transferred to a 50 mL centrifuge tube with 9 mL of methanol and incubated in a 65 °C water bath for 30 min and vortexed for 5 s every 10 min. Then 0.5 mL 10 M KOH was added, vortexed vigorously for about 1 min, and incubated at 65 °C for 2 h with 5 s vortexes every hour. After cooling to room temperature in a cold tap water bath, 0.5 mL concentrated H_2SO_4 (18.4 M) was added and the same 65 °C incubation was repeated. After cooling to room temperature, 4 mL hexane was added to the tube and vortexed for 1 min to extract the lipids, then centrifuged at 1,351× g for 5 min. The top hexane layer was then transferred to a clean 15 × 125 mm glass tube. This extraction step was repeated with another 4 mL hexane and the top hexane layer was transferred to the same glass tube. The extracted lipids were dried under nitrogen gas, and resuspended in 0.5 mL hexane for lipid composition analysis.

2. Supplementary tables

Table S1 Concentration of common components in normal YNB and synthetic seawater.

Component	Concentration in YNB (g L^{-1})	Concentration in seawater (g L^{-1})
NaCl	0.1	23.9
Na_2SO_4	-	4
$MgSO_4$	0.5	-
$MgCl_2{\cdot}6H_2O$	-	10.8
$CaCl_2{\cdot}2H_2O$	0.1	1.5
KH_2PO_4	1	-
KI	0.0001	-
KCl	-	0.7
KBr	-	0.1
H_3BO_3	0.0005	-

Table S2 List of strains used.

Name[a]	Description
YLH2	Unevolved ancestral strain (GSY1136 YIplac211YB/I/E* Δ*CTT1*)
SM14	Isolated hyper-producer from evolved population 1
SM11	Isolated hyper-producer from evolved population 1
SM12	Isolated hyper-producer from evolved population 1
SM13	Isolated hyper-producer from evolved population 1
SM22	Isolated hyper-producer from evolved population 2
SM24	Isolated hyper-producer from evolved population 2

[a] Strains used in this work were from our previous work [1].

Table S3 Highest β-carotene production in different conditions.

Strains and conditions	β-carotene production (mg g^{-1} DCW)	Reference
Saccharomyces cerevisiae SM14 in nutrient reduced media made with water, cultured in shaking incubator	8.25 ± 0.51	This work
Saccharomyces cerevisiae SM14 in nutrient reduced media made with 1/3× seawater, cultured in shaking incubator	17.37 ± 1.12	This work
Saccharomyces cerevisiae SM14 in YPD media, cultured in shaking incubator	18	[1]
Saccharomyces cerevisiae SM14 in YNB media, cultured in bioreactor	25.52 ± 2.15	[2]
Saccharomyces cerevisiae YB/I/E in YNB media, cultured in shaking incubator	5.9	[3]
Rhodotorula glutinis NCIM 3353 in YNB media, cultured in shaking incubator	5.38	[4]

Table S4 Comparison of β-carotene measurement between spectrophotometric assay and HPLC.

Samples	spectrophotometric assay (mg L^{-1})	HPLC (mg L^{-1})
1	3.87	3.27
2	1.41	0.91

3. Supplementary figures

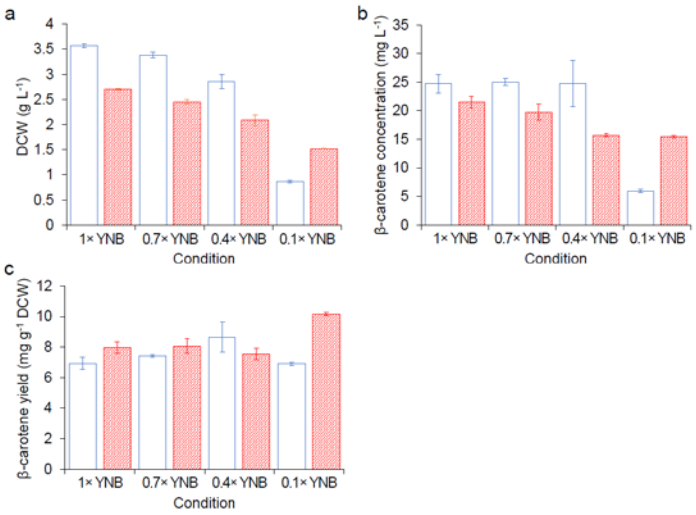

Figure S1 Effect of YNB concentration on biomass formation and β-carotene production of SM14 in freshwater ( ) and seawater ( ) after 72 h incubation. (a) Biomass (DCW: dry cell weight). (b) β-carotene concentration. (c) β-carotene yield. Error bars are standard deviations.

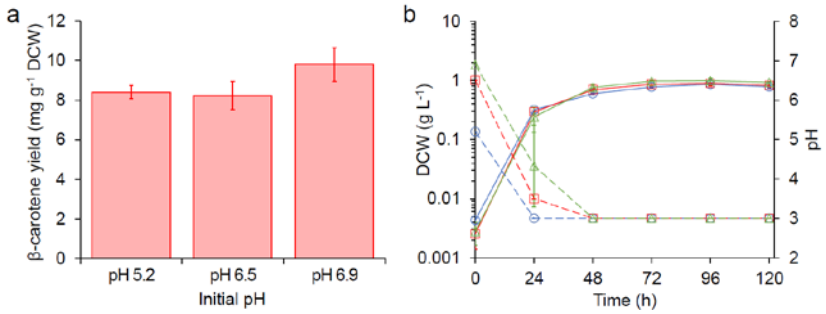

Figure S2 β-carotene production after 120 h (a), cell growth (solid lines) (DCW: dry cell weight) and pH change (dashed lines) (b) of SM14 in freshwater with different initial pH, ( ) initial pH 5.2, ( ) initial pH 6.5, ( ) initial pH 6.9. Error bars are standard deviations

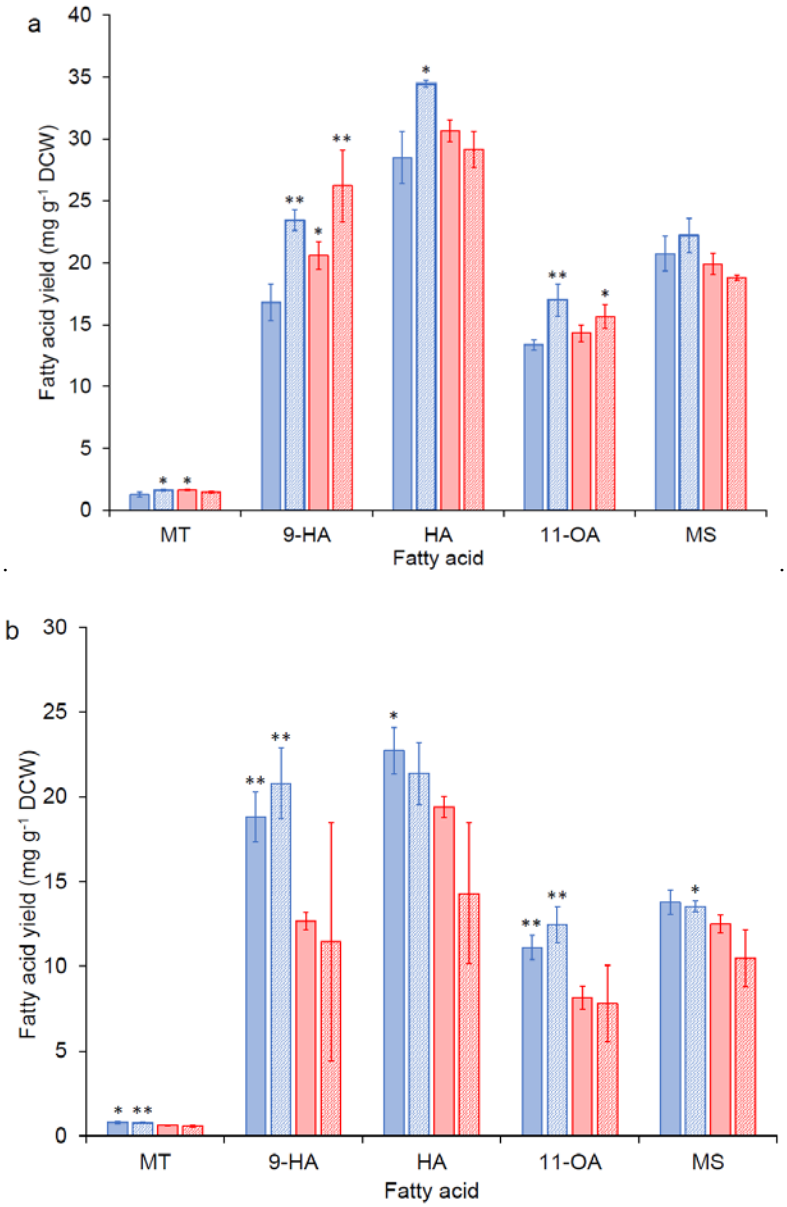

Figure S3 Individual fatty acid yield. (a) ( ) C:N = 8.8 in freshwater, ( ) C:N = 50 in freshwater, ( ) C:N = 8.8 in freshwater with 0.05 M NaCl, ( ) C:N = 50 in freshwater with 0.05 M NaCl; (b) ( ) C:N = 8.8 in 1/3× seawater, ( ) C:N = 50 in 1/3× seawater, ( ) C:N = 8.8 in seawater, ( ) C:N = 50 in seawater. Error bars are standard deviations. *: Statistically significantly different from C:N = 8.8 in freshwater (a) or seawater (b) (*p-value < 0.05, **p-value < 0.01). MT: Methyl tetradecanoate; 9-HA: 9-Hexadecenoic acid; HA: Hexadecanoic acid; 11-OA: 11-Octadecenoic acid; MS: Methyl stearate.

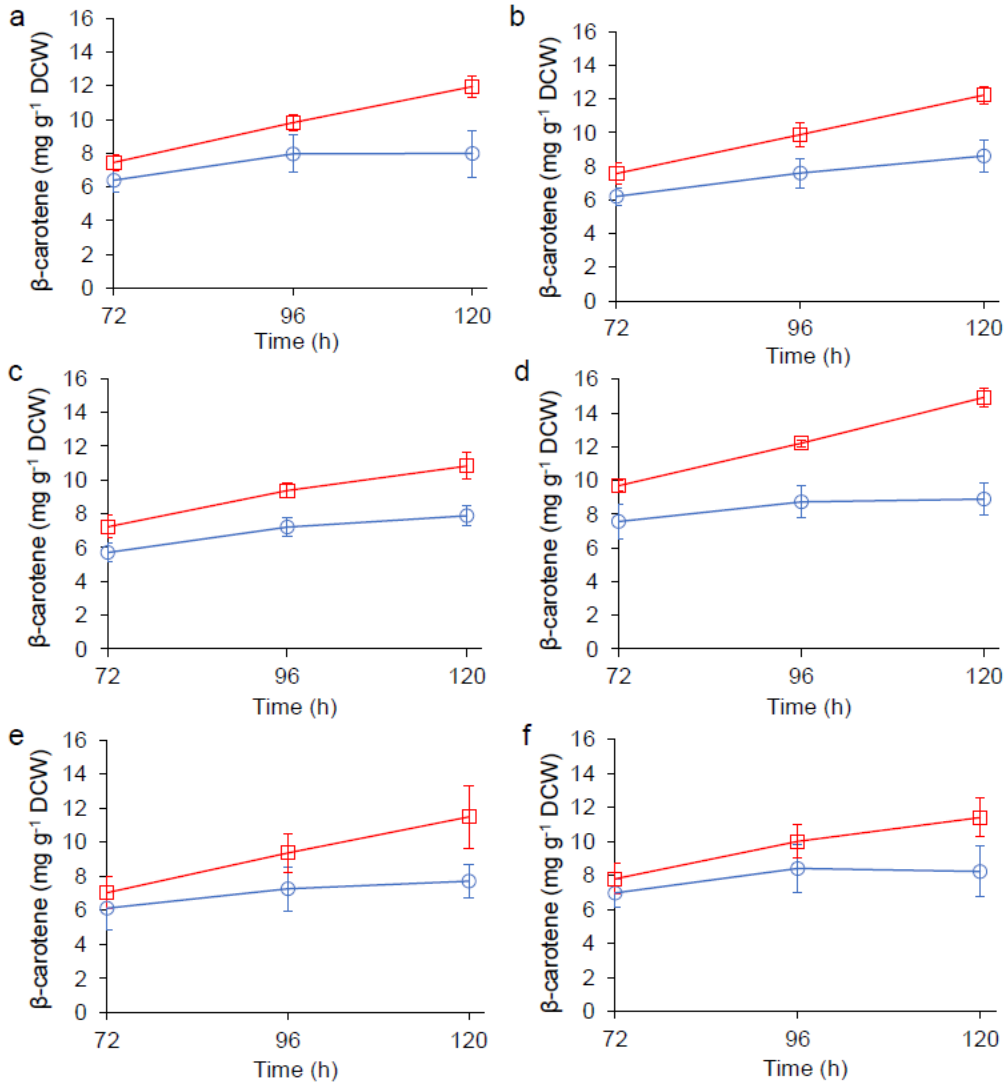

Figure S4 β-carotene production of carotenogenic strains in 0.1× YNB with freshwater ( ) or 1/3× seawater ( ). (a) SM11. (b) SM12. (c) SM13. (d) SM22. (e) SM24. (f) YLH2. Error bars are standard deviations.

Reference

1. Reyes, L.H.; Gomez, J.M.; Kao, K.C. Improving carotenoids production in yeast via adaptive laboratory evolution. *Metab Eng* **2014**, *21*, 26-33.
2. Olson, M.L.; Johnson, J.; Carswell, W.F.; Reyes, L.H.; Senger, R.S.; Kao, K.C. Characterization of an evolved carotenoids hyper-producer of *Saccharomyces cerevisiae* through bioreactor parameter optimization and raman spectroscopy. *J Ind Microbiol Biotechnol* **2016**, *43*, 1355-1363.
3. Verwaal, R.; Wang, J.; Meijnen, J.P.; Visser, H.; Sandmann, G.; van den Berg, J.A.; van Ooyen, A.J. High-level production of beta-carotene in *Saccharomyces cerevisiae* by successive transformation with carotenogenic genes from *Xanthophyllomyces dendrorhous*. *Appl Environ Microbiol* **2007**, *73*, 4342-4350.
4. Bhosale, P.; Gadre, R.V. Production of beta-carotene by a *Rhodotorula glutinis* mutant in sea water medium. *Bioresour Technol* **2001**, *76*, 53-55.