Supporting Information

Multidimensional Engineering of Komagataella phaffii for Efficient Synthesis of Retinal

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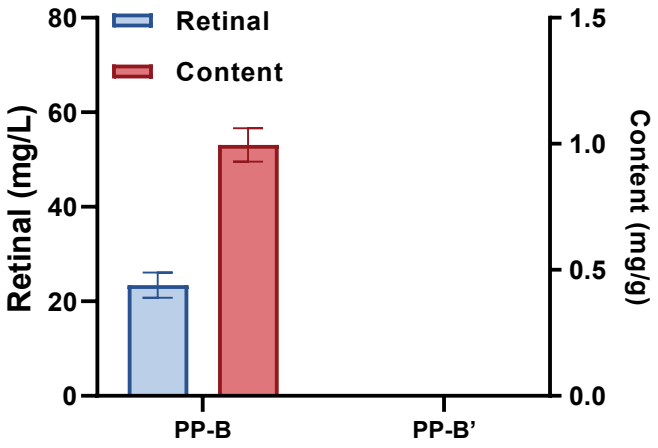

Figure S1. Production of retinal and content of engineered PP-B and PP-B'.

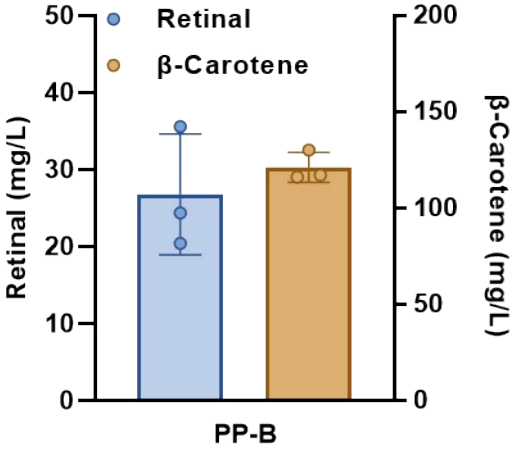

Figure S2. Production of retinal and β-carotene of engineered strain PP-B.

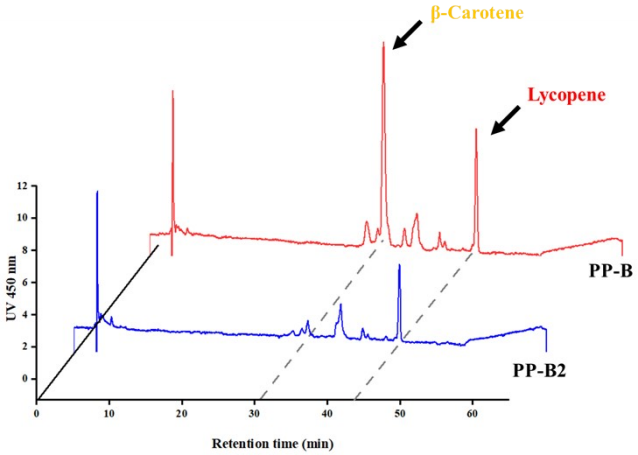

Figure S3. HPLC analysis of the carotenoid profile of PP-B and PP-B2.

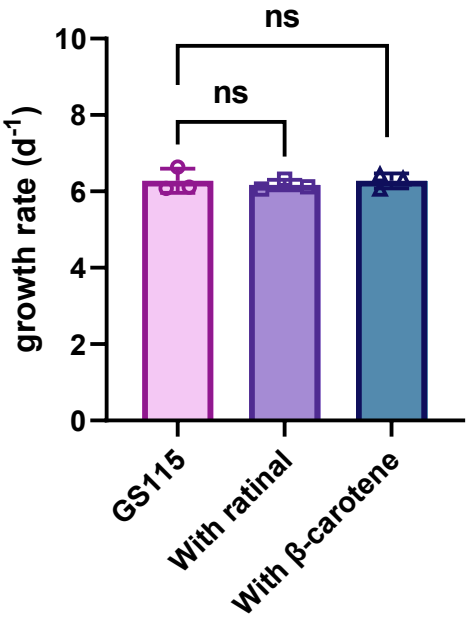

Figure S4. Effects of additional supplementation of β-carotene and retinal on the growth rate of *K. phaffii* GS115.

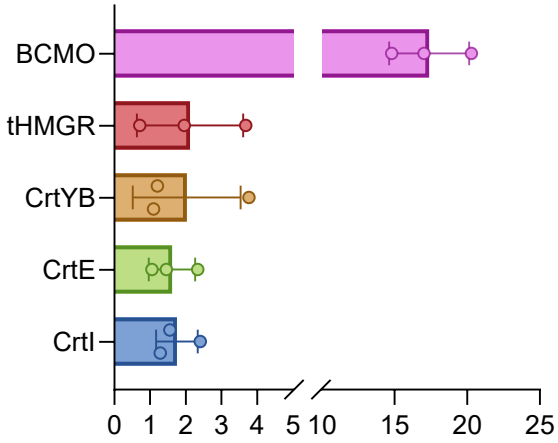

Figure S5. Key gene validation analysis of PP-B compared with PP-B2.

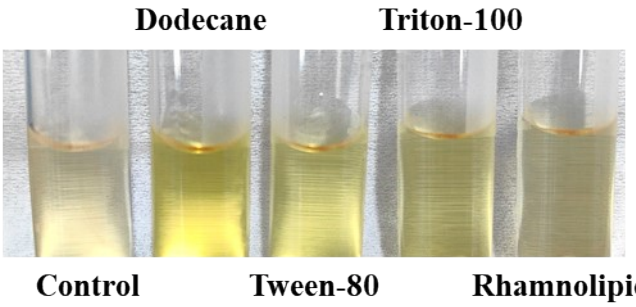

Figure S6. Color differences in the supernatant after addition of different surfactants.

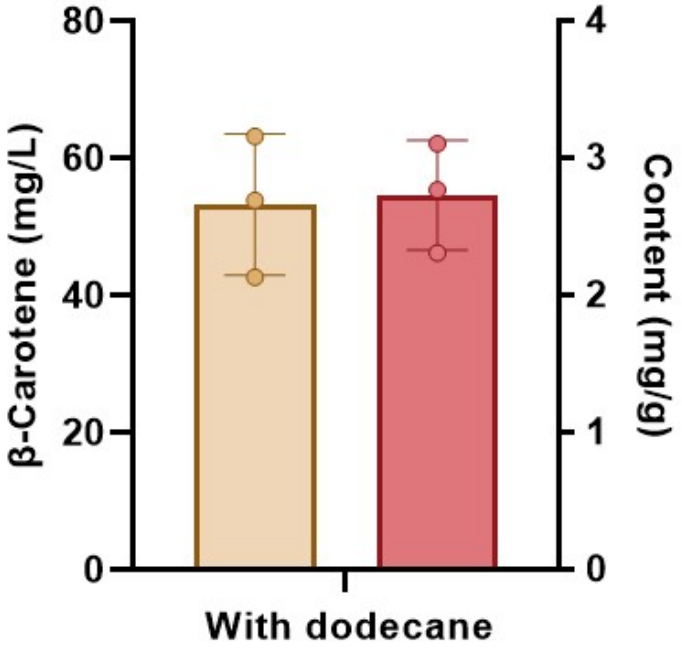

Figure S7. The production and content of β-carotene with adding dodecane.

Table S1. Primers Used in This Study.

Primers	**Sequence**
crtI-F	GGTCTCCCATGACTGCTTTGGCCTA
crtI-R	GGTCTCGAAGCTTACTGACCCTCCCAAC
crtE-F	GGTCTCCCATGGGCAAAGAGAAGGA
crtE-R	GGTCTCAAAGCGAATTCTTAGAAGG
crtYB-F	GGTCTCCCATGGACTACGCCAACAT
crtYB-R	GGTCTCGAAGCTTACAGTGGGATGT
tHMGR-F	GGTCTCCCATGGACCAATTGGTGAA
tHMGR-R	GGTCTCGAAGCTTAGGATTTAATGC
BLH-F	GGTCTCCCATGGGACTCATGCTAAT
BLH-R	GGTCTCGAAGCTCAATTTTTAATCTTA

Table S2. Plasmids Used in This Study.

Plasmid	Description	Source
BB1-23	Integrative vector with the Kan^R marker	Purchase
BB1-23-crtI	*crtI*, Kan^R	This study
BB1-23-crtE	*crtE*, Kan^R	This study
BB1-23-crtYB	*crtYB*, Kan^R	This study
BB1-23-tHMGR	*tHMGR*, Kan^R	This study
BB1-23-BLH	*blh*, Kan^R	This study
BB1-23-carRP	*carRP,* Kan^R	This study
BB1-23-carB	*carB*, Kan^R	This study
BB1-23-BCO1	BCO1, Kan^R	This study
BB2-AB	Integrative vector with the Amp^R marker	Purchase
BB2-BC	Integrative vector with the Amp^R marker	Purchase
BB2-CD	Integrative vector with the Amp^R marker	Purchase
BB2-DE	Integrative vector with the Amp^R marker	Purchase
BB2-EF	Integrative vector with the Amp^R marker	Purchase
BB2-AB-crtI	P_{GPM1}-*crtI*-T_{ScCYC1}, Amp^R	This study
BB2-BC-crtE	P_{PDC1}-*crtE*-T_{RPP1B}, Amp^R	This study
BB2-CD-crtYB	P_{MDH3}-*crtYB*-T_{RPS2}, Amp^R	This study
BB2-DE-tHMGR	P_{ADH2}-*tHMGR*-T_{RPL2A}, Amp^R	This study
BB2-CD-tHMGR	P_{ADH2}-*tHMGR*-T_{RPL2A}, Amp^R	This study
BB2-EF-BLH	P_{GAP}-*blh*-T_{RPS25A}, Amp^R	This study

BB2-EF-BCO1	P_{GAP}-BCO1-T_{RPS25A}, Amp^R	This study
BB2-AB-carRP	P_{GPM1}-*carRP*-T_{ScCYC1}, Amp^R	This study
BB2-BC-carB	P_{PDC1}-*carB*-T_{RPP1B}, Amp^R	This study
BB3-aK-AF	Integrative vector with the Kan^R marker	Purchase
BB3-aK-AD-crtIEYB	P_{GPM1}-*crtI*-T_{ScCYC1}, P_{PDC1}-*crtE*-T_{RPP1B}, P_{MDH3}-*crtYB*-$T_{RPS2,}$ Kan^R	This study
BB3-aK-AC-carRP/B	P_{GPM1}-*carRP*-T_{ScCYC1}, P_{PDC1}-*carB*-T_{RPP1B}, Kan^R	This study
BB3-aK-AE-crtIEYBt	P_{GPM1}-*crtI*-T_{ScCYC1}, P_{PDC1}-*crtE*-T_{RPP1B}, P_{MDH3}-*crtYB*-$T_{RPS2,}$ P_{ADH2}-*tHMGR*-$T_{RPL2A,}$ Kan^R	This study
BB3-aK-AF-crtIEYBtBLH	P_{GPM1}-*crtI*-T_{ScCYC1}, P_{PDC1}-*crtE*-T_{RPP1B}, P_{MDH3}-*crtYB*-T_{RPS2}, P_{ADH2}-*tHMGR*-T_{RPL2A}, P_{GAP}-*blh*-T_{RPS25A}, Kan^R	This study
BB3-aK-AF-crtIEYBtBCO1	P_{GPM1}-*crtI*-T_{ScCYC1}, P_{PDC1}-*crtE*-T_{RPP1B}, P_{MDH3}-*crtYB*-T_{RPS2}, P_{ADH2}-*tHMGR*-T_{RPL2A}, P_{GAP}-BCO1-T_{RPS25A}, Kan^R	This study
BB2-AB-BLH	P_{GAP}-*blh*-T_{RPS25A}, Amp^R	This study
BB2-BC-GND2	P_{GPM1}-*GND2*-T_{RPP1B}, Amp^R	This study
BB2-CD-POS5	P_{ADH2}-*POS5*-T_{RPS2}, Amp^R	This study
BB2-DE-ZWF1	P_{TEF1}-*ZWF1*-T_{RPL2A}, Amp^R	This study
BB3-Z4-intE12-BLH-GND2-	P_{GAP}-*blh*-T_{RPS25A}, P_{GPM1}-*GND2*-T_{RPP1B}, P_{ADH2}-*POS5*-T_{RPS2}, P_{TEF1}-*ZWF1*-T_{RPL2A}, $Bleo^R$	This study

POS5-ZWF1		
BB3-Z5-intE12-BLH-GND2-POS5-ZWF1-DGA1	P_{GAP}-*blh*-T_{RPS25A}, P_{GPM1}-*GND2*-T_{RPP1B}, P_{ADH2}-*POS5*-T_{RPS2}, P_{TEF1}-*ZWF1*-T_{RPL2A}, P_{RPP1B}-*DGA1*-T_{RPS25A}, $Bleo^R$	This study
BB1-23-ACL	ACL, Kan^R	This study
BB1-23-ACS	ACS, Kan^R	This study
BB1-23-PTA	PTA, Kan^R	This study
BB1-23-PK	PK, Kan^R	This study
BB2-AB-ACL	P_{GAP}-ACL-T_{RPS25A}, Amp^R	This study
BB2-BC-ACS	P_{MDH3}-ACS-T_{RPS2}, Amp^R	This study
BB2-AB-PTA	P_{MDH3}-PTA-T_{RPS2}, Amp^R	This study
BB2-BC-PK	P_{GAP}-PK-T_{RPS25A}, Amp^R	This study
BB3rN-AC-ACL-ACS	P_{GAP}-ACL-T_{RPS25A}, P_{MDH3}-ACS-T_{RPS2}, Nat	This study
BB3rN-AC-PTA-PK	P_{MDH3}-PTA-T_{RPS2}, P_{GAP}-PK-T_{RPS25A}, Nat	This study
BB1-23-CK	CK, Kan^R	This study
BB1-23-IPK	IPK, Kan^R	This study
BB2-CD-IPK	P_{ADH2}-IPK-T_{RPS2}, Amp^R	This study
BB2-DE-CK	P_{GPM1}-CK-T_{RPL2A}, Amp^R	This study
BB3rN-AE-ACL-	P_{GAP}-ACL-T_{RPS25A}, P_{MDH3}-ACS-T_{RPS2}, P_{ADH2}-	This study

ACS-IPK-CK	IPK-T_{RPS2}, P_{GPM1}-CK-T_{RPL2A}, Nat	
BB3rN-AE-PTA-PK-IPK-CK	P_{MDH3}-PTA-T_{RPS2}, P_{GAP}-PK-T_{RPS25A}, P_{ADH2}-IPK-T_{RPS2}, P_{GPM1}-CK-T_{RPL2A}, Nat	This study
BB3eH-AC-EGFP-PEX15-mCherry-ePTS1	P_{GAP}-EGFP-PEX15-T_{ScCYC1}, P_{GCW14}-mCherry-ePTS1-T_{RPL2A}	This study
BB3rN-AF-PTA-PK-IPK-CK-blh-PEX15	P_{MDH3}-PTA-T_{RPS2}, P_{GAP}-PK-T_{RPS25A}, P_{ADH2}-IPK-T_{RPS2}, P_{GPM1}-CK-T_{RPL2A}, P_{GAP}-blh-PEX15-T_{ScCYC1}, Nat	This study
BB1-23-VHb	VHb, Kan^R	This study
BB1-23-Yor1	Yor1, Kan^R	This study
BB1-23-Snq2	Snq2, Kan^R	This study
BB1-23-Pdr5	Pdr5, Kan^R	This study
BB1-23-Pdr10	Pdr10, Kan^R	This study
BB1-23-Pdr12	Pdr12, Kan^R	This study
BB1-23-Pdr15	Pdr15, Kan^R	This study
BB3eH-14	Integrative vector with the Hygromycin B marker	Purchase
BB3eH-14-VHb	P_{GCW14}-VHb-T_{RPP17B}, Hygromycin B	This study
BB3eH-14-Yor1	P_{GAP}-Yor1-T_{ScCYC1}, Hygromycin B	This study
BB3eH-14-Snq2	P_{GAP}-Snq2-T_{ScCYC1}, Hygromycin B	This study
BB3eH-14-Pdr5	P_{GAP}-Pdr5-T_{ScCYC1}, Hygromycin B	This study
BB3eH-14-Pdr10	P_{GAP}-Pdr10-T_{ScCYC1}, Hygromycin B	This study

BB3eH-14-Pdr12	P_{GAP}-Pdr12-T_{ScCYC1}, Hygromycin B	This study
BB3eH-14-Pdr15	P_{GAP}-Pdr15-T_{ScCYC1}, Hygromycin B	This study
BB2-AB-Pdr5	P_{GAP}-Pdr5-T_{ScCYC1}, Amp^R	This study
BB2-BC-VHb	P_{GCW14}-VHb-T_{RPP17B}, Amp^R	This study
BB3eH-AC-Pdr5-VHb	P_{GAP}-Pdr5-T_{ScCYC1}, P_{GCW14}-VHb-T_{RPP17B}, Hygromycin B	This study

Table S3. Comparison of fermentation substrates and yields between the strains constructed in this study and previous studies.

Strains	Substrate	Yields	References
E. coli	Glycerol and arabinose	109 mg/L retinoid	[1]
E. coli	Glycerol	136 mg/L retinoid	[2]
S. cerevisiae	Glucose and xylose	97.09 mg/L retinol	[3]
S. cerevisiae	Glucose and ethanol	2479.34 mg/L retinol	[4]
Y. lipolytica	Glucose	4.86 g/L retinol	[5]
Y. lipolytica	Glucose	8.92 g/L retinol	[6]
E. coli	Glucose	245.73 mg/L retinal	[7]
S. cerevisiae	Glucose	69.13 mg/L retinal	[8]
S. cerevisiae	Glucose	638.12 mg/L retinal	[9]
K. phaffii	Glucose	764.22 mg/L retinal	This study
K. phaffii	Methanol	1406.69 mg/L retinal	This study

References:

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