

Supplementary Information

Expression of VHb improved lipid production in *Rhodospiridium toruloides*

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Table S1. Strains and plasmids used in this study

Stain	Relevant description	Resource
<i>E. coli</i> DH5α	<i>F- λ- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZDM15 D (lacZYA-argF) U169, hsdR17(rK- mK+)</i>	TaKaRa
<i>R. toruloides</i> AS 2.1389 (4#)	Diploid strain	CGMCC
<i>A. tumefaciens</i> AGL1	<i>AGL0 recA::bla pTiBo542DT Mop + CbR</i>	[22]
<i>R. toruloides</i> 4#-13	<i>R. toruloides</i> CGMCC 2.1389, P _{PGK} - <i>hyg</i> -T _{NOS} -P _{LDPI} - <i>vgb</i> -T _{HSP}	This study
<i>R. toruloides</i> 4#-14	<i>R. toruloides</i> CGMCC 2.1389, P _{PGK} - <i>hyg</i> -T _{NOS} -P _{LDPI} - <i>vgb</i> -T _{HSP}	This study
<i>R. toruloides</i> 4#-15	<i>R. toruloides</i> CGMCC 2.1389, P _{PGK} - <i>hyg</i> -T _{NOS} -P _{LDPI} - <i>vgb</i> -T _{HSP}	This study
Plasmid		
pZPK		Our lab
pUC57- <i>vgb</i>	Amp ^R	This study
pZPK-P _{PGK} - <i>hyg</i> -T _{NOS} -P _{GPD} - <i>vgb</i> -T _{HSP}	Kan ^R	This study
pZPK-P _{PGK} - <i>hyg</i> -T _{NOS} -P _{LDPI} - <i>vgb</i> -T _{HSP}	Kan ^R	This study

Table S2. Primer used in this study

Primer name	Sequence (5' - 3')	Description
NcoI-F	CACCCCATGGATGCTCGACCAGCAGA	Amplification of <i>vgb</i> from pUC57
Ble -P2A-VHb-R	CTAGTCCTGCTCCTCGGCCACGAA	
Tnos-LDP1-RF1-f	GTGTCATCTATGTTACTAGATCGGGCCTGGATCCTCTA GAGTCGCAGTTTTGCAAGGTCACGCTC	Amplification of promoter LDP1
Tnos-LDP1-RF1-r	GATGATGTTGATCGTCTGCTGGTCGAGCATGTCCATGG GATATCGGGGTAGTCGAGCGCCTGTGTTGCACG	
Ldp1-420-F	CGAGACCGCCATCCACCGCGTG	Amplification of <i>vgb</i> from gDNA
Thsp-129-R	GCAGTACTGCAGGCGTGGAGAACG	

Table S3. Quantifications of lipid droplets.

Lipid drops number (4#)	4#/area	Lipid drops number (4#-13)	4#-13/area
1	148	1	395
2	205	2	283
3	180	3	325
4	250	4	330
6	213	5	313
7	100	6	262
Mean area	182		318

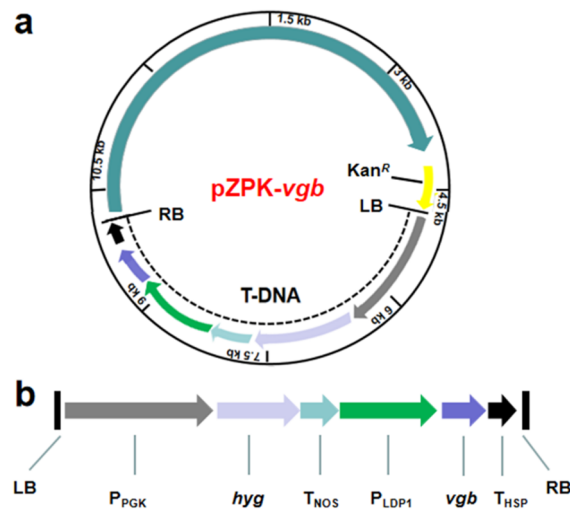


Figure S1. Vector construction. **a)** The vector pZPK-P_{PGK}-hyg-T_{NOS}-P_{LDP1}-vgb-T_{HSP} was used for ATMT of *R. toruloides* and contains the kanamycin resistance gene (Kan^R) and transfer DNA region (T-DNA). **b)** Enlargement of the T-DNA region that is delimited by the right (RB) and left border (LB) and contains the *Vitreoscilla* hemoglobin gene (*vgb*) under control of the constitutive promoter P_{LDP1}, the hygromycin resistance gene under control of constitutive promoter P_{PGK}.

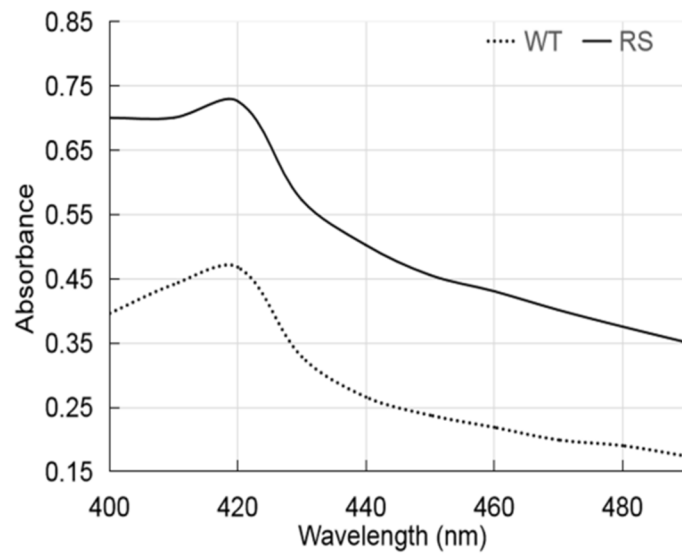


Figure S2. CO differential chromatograms of *R. toruloides* 4#-13 (RS) and the wild type stain (WT).

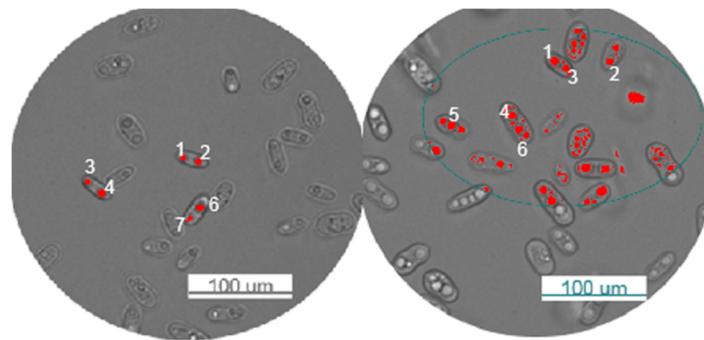


Figure S3. Cell morphological differences between *R. toruloides* 4# (left) and *R. toruloides* 4#-13 (right). Cells were collected from cultures in 3-L fermenter and observed by the EVOS microscope.