Supplementary information

Octanoic production in *Saccharomyces cerevisiae*: Investigation of new precursor supply engineering strategies and intrinsic limitations

Florian Wernig[1], Leonie Baumann[1] Eckhard Boles[1] & Mislav Oreb*[1]

[1]Institute of Molecular Biosciences, Faculty of Biological Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany

*Corresponding author
Dr. Mislav Oreb
Goethe University Frankfurt
Institute of Molecular Biosciences
Max-von-Laue Straße 9
60438 Frankfurt
Germany
Telephone +49 (0)69 798 29331
Telefax +49 (0)69 798 29527
E-Mail m.oreb@bio.uni-frankfurt.de

Table S1: Oligonucleotides used in this study

Primer Name	Sequence 5'-3'	Application
FWP322	CTGTCACCGTCAGAAAAATATGTCAATGAG GCAAGAACCGGGCTGGGCGATCTTCCTTG	Amplification of *COX9p* with overhangs to *PGI1* locus to replace *PGI1p*
FWP323	GCCAGTTTGAAGTTAGTGAATGAGTTATTG GACATGTCTGTGTAAGTCGCTTGTAGTTAG	
SBP176	CTCTATTCCACGAGGCATTC	Amplification of *HXT7p*$^{-1--392}$ with overhangs to *ZWF1* locus to replace *ZWF1p*
FWP327	ACCCGTGTACATAAGCGTGAAATCACCACA AACTGTGTGTAGCTCGTAGGAACAATTTCG	
FWP331	ACACATTACGTTAGCAAAAGCAACAATAAC AAACACAACCGGCATCCTGCAACTGTTCTG TGGAGCTATTAAATCTTTAT	Donor DNA for deletion of *ARE2*
FWP332	ATAAAGATTTAATAGCTCCACAGAACAGTT GCAGGATGCCGGTTGTGTTTGTTATTGTTGC TTTTGCTAACGTAATGTGT	
FWP337	TAAGGAAACGCAGAGGCATACAGTTTGAAC AGTCACATAATAATGAATTCATTGGAAAAC ACAAAATATGTTAGAATAAA	Donor DNA for deletion of *DGA1*
FWP338	TTTATTCTAACATATTTTGTGTTTTCCAATG AATTCATTATTATGTGACTGTTCAAACTGTA TGCCTCTGCGTTTCCTTA	
FWP342	TTGATTGCCATTTTTTTCTTTCCAAGTTTCCT TGTTATAAAATTAAAGTAGCAGTACTTC	Amplification of xPK-PTA cassette with overhangs for *GPP1* locus
FWP343	TTTATTTTTAGCGTAGTAGTTTTATCAAAAA AATAAAAGAAAACACCCATGAACCACAC	
FWP362	TTGAAGAAACATGAAATTGCCCAGTATTCT TAACCCAAAATGGATATTGATCTAGATGG	Amplification of *PFK1p-RPE1-RPE1t* with overhangs to *URA3* locus
FWP363	AATCATTACGACCGAGATTCCCGGGTAATA ACTGGAAAAATATAAGGATGAGAAAGTG	
FWP366	GTCGACGGTATCGATAAGCTTGATATCGAA TTCCTGCAGTTGATAGGTCAAGATCAATG	Amplification of *TEF2p* with overhangs to prS52-K and *ZWF1* for cloning of FWV169
FWP367	ATTTTTTTCGAATTTGACGGGGCCTTCACTC ATGTTTAGTTAATTATAGTTCGTTGAC	
FWP368	CTTGTTTTTAGAATATACGGTCAACGAACT ATAATTAACTAAACATGAGTGAAGGCCC	Amplification of *ZWF1-ZWF1t* with overhangs to *TEF2p* and pRS52-K for cloning of FWV169
FWP369	AAATTTGTATTTGTAGAGTGCATCCTATATA TTCAATTCATATTTTATCTCTTTTTTTTTTTT TTTTTTC	
FWP170	AAAAAAAAAAGAGATAAAATATGAATTGA ATATATAGGATGCACTCTACAAATAC	Amplification of *SOL3-SOL3t* with overhangs to *CCW12p* and pRS52-K for cloning of FWV169
FWP171	AGAAATTAATCTTCTGTCATTCGCTTAAACA CTATATCAATAAATGGTGACAGTCGGTG	
FWP172	AGCCCTCTCAGAAAACACACCGACTGTCAC CATTTATTGATATAGTGTTTAAGCGAATG	Amplification of *CCW12p* with overhangs to prS52-K and *SOL3* for cloning of FWV169
FWP173	TTTTGAGCCTCCATGTCTCTGAAGAACTCCC TGTTGGCAAGGCACCCATGAACCACAC	
FWP174	ATCTGAACTGCCCCTTTTGGACTAACCGTGT GGTTCATGGGTGCCTTGCCAACAGGG	Amplification of *TEF1p* with overhangs to prS52-K and *GND1* for cloning of FWV169
FWP175	AAACCAATCAAACCGAAATCAGCAGACATT TTGTAATTAAAACTTAGATTAGATTGC	
FWP176	GAAAGAAAGCATAGCAATCTAATCTAAGTT TTAATTACAAAATGTCTGCTGATTTCGG	Amplification of *GND1-GND1t* with overhangs to *TEF1p* and pRS52-K for cloning of FWV169
FWP177	TTAACCCTCACTAAAGGGAACAAAAGCTGG AGCTCCACCGCGGTCTACTCTACTTCTATCA	

	TGATAATAG	
FWP418	TCATTATAGAAATCATTACGACCGAGATTC CCGGGTAATAACTGGGCAAAACGTAGGG	Amplification of *ADH2p* with overhangs to *ALD6* and *URA3* for integration
FWP402	GGTTCAGCAGTGTCAAAGTGTAGCTTAGTC ATTGTGTATTACGATATAGTTAATAG	
FWP410	AATCAACTATCAACTATTAACTATATCGTA ATACACAATGACTAAGCTACACTTTGAC	Amplification of *ALD6-ALD6t* with overhangs to *ADH2p* and *URA3* integration
FWP419	TTGAAGAAACATGAAATTGCCCAGTATTCT TAACCCAATCCACGTTAGTTTTCTTTGG	
FWP413	TTTTGCATTGCCTTATCTTTTGCCGCCAGAA GAAACAAGGTGACGACGGATGAATATGTTG ACAGTCTAGCAAACAGTAG	Donor DNA for deletion of *ALD2*
FWP414	CTACTGTTTGCTAGACTGTCAACATATTCAT CCGTCGTCACCTTGTTTCTTCTGGCGGCAAA AGATAAGGCAATGCAAAA	
FWP415	AACCCTTAATATAACTTCGTATAATGTATGC TATACGAAGTTATCAGCGACATGGAGGC	Amplification of *kanMX4* with overhangs to pRS42-H for replacement of *hphNT*
FWP416	ATATCACCTAATAACTTCGTATAGCATACA TTATACGAAGTTATGACACTGGATGGCGG	

Table S2: Plasmids used in this study

Laboratory stock code	Plasmid name	Relevant elements	Reference
ASB13	pRS41-H	*CEN6/ARS4,, AmpR, hphNT1*	Taxis and Knop., 2006
ASB16	pRS42-H	*2micron, AmpR, hphNT1*	Taxis and Knop., 2006
ASB20	pRS52-K	*2micron, AmpR, kanMX4,*	Boles lab stock
ASB22	pRS62-H	*2micron, AmpR, hphNT1*	Boles lab stock
ASB23	pRS62-K	*2micron, AmpR, kanMX4,*	Farwick et al., 2014
AB02	pRCC-N-POX1	*2µ, AmpR, natNT2, ROX3p-opt.CAS9^{Sp}-CYC1t, SNR52p-gRNA[POX1]-SUB4t*	This study
MR02	pRCC-K-URA3	*2µ, AmpR, kanMX4, ROX3p-opt.CAS9^{Sp}-CYC1t, SNR52p-gRNA[URA3]-SUB4t*	Boles lab stock
HDV10	pHD8	*2µ, AmpR, kanMX4, PFK1p-RPE1-RPE1t*	Demeke et al., 2013
FWV169	prRS52-K-oxPPP	*2µ, AmpR, kanMX4, TEF2p-ZWF1-ZWF1t, CCW12p-SOL3-SOl3t, TEF1p-GND1-GND1t*	This study
FWV133	pRS313H-fusFAS^{RK}	*CEN6/ARS4, AmpR, hphNT1, FAS1p-FAS1^{R1834K}-FAS2-FAS2t*	Wernig et al., 2020
SHV61	pRS315-fusFAS^{RK}	*CEN6/ARS4, AmpR, LEU2, FAS1p-FAS1^{R1834K}-FAS1t, FAS2p-FAS2-FAS2t*	Wernig et al., 2020
TWRV1	pRS42-H-^{Se}ACS-ALD6	*2µ, AmpR, hphNT1, PGI1p-ALD6-RPL15At, PFK1p-^{Se}ACS^{L641P}-DIT1t*	This study
FWV171	pRS42-K-^{Se}ACS-ALD6	*2µ, AmpR, kanMX, PGI1p-ALD6-RPL15At, PFK1p-^{Se}ACS^{L64}-DIT1t*	This study
VSV111	pRCC-K-ALD6	*2µ, AmpR, kanMX4, ROX3p-opt.CAS9^{Sp}-CYC1t, SNR52p-gRNA[ALD6]-SUB4t*	Schadeweg & Boles, 2016
ISOV115	pRS42-H-ADH2	*2µ, AmpR, hphNT1, HXT7p$^{-1--392}$-ADH2-FBA1t*	Brat & Boles, 2012
FWV156	pRCC-K-P$_{PGI1}$	*2µ, AmpR, kanMX4, ROX3p-opt.CAS9^{Sp}-CYC1t, SNR52p-gRNA[pPGI1]-SUB4t*	This study

FWV157	pRCC-K-P_{ZWF1}	*2μ, AmpR, kanMX4, ROX3p-opt.CAS9^{Sp}-CYC1t, SNR52p-gRNA[pZWF1]-SUB4t*	This study
FWV158	pRCC-K-ARE2	*2μ, AmpR, kanMX4, ROX3p-opt.CAS9^{Sp}-CYC1t, SNR52p-gRNA[ARE2]-SUB4t*	This study
FWV159	pRCC-K-DGA1	*2μ, AmpR, kanMX4, ROX3p-opt.CAS9^{Sp}-CYC1t, SNR52p-gRNA[DGA1]-SUB4t*	This study
pB14	pYTK001-^{Ca}xPK-B14	*CamR,* ^{Ca}xPK	Arun S. Rajkumar
pB15	pYTK001-^{Bs}xPTA-B15	*CamR,* ^{Bs}xPTA	Arun S. Rajkumar
pB20	pYTK001-^{Se}EutD-B20	*CamR,* ^{Se}EutD	Arun S. Rajkumar
FWV163	pRS62-H-^{Ca}xPK	*2μ, AmpR, hphNT1, HXT7p$^{-1—392}$-^{Ca}xPK-FBA1t*	This study
FWV164	cGG-^{Bs}PTA	*HHF2p-^{Bs}PTA-SSA1t*	This study
FWV165	cGG-^{Se}EutD	*HHF2p-^{Se}EutD-SSA1t*	This study
FWV168	pRCC-N-GPP1	*2μ, AmpR, natNT2, ROX3p-opt.CAS9^{Sp}-CYC1t, SNR52p-gRNA[GPP1]-SUB4t*	This study

Sequences of relevant genes.

>^{Ca}xPK

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>^{Bs}PTA

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>^{Se}EutD

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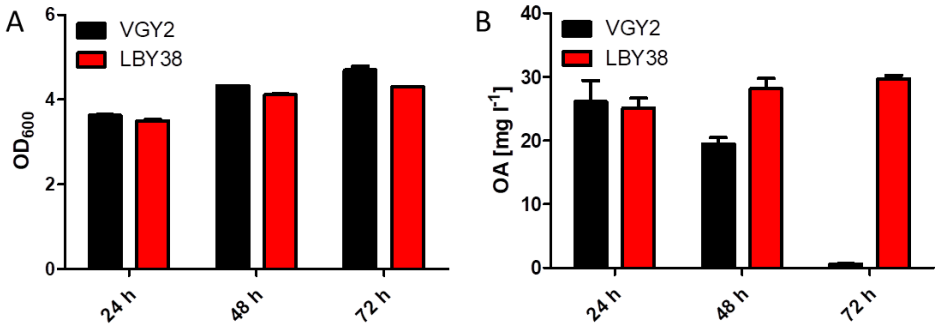
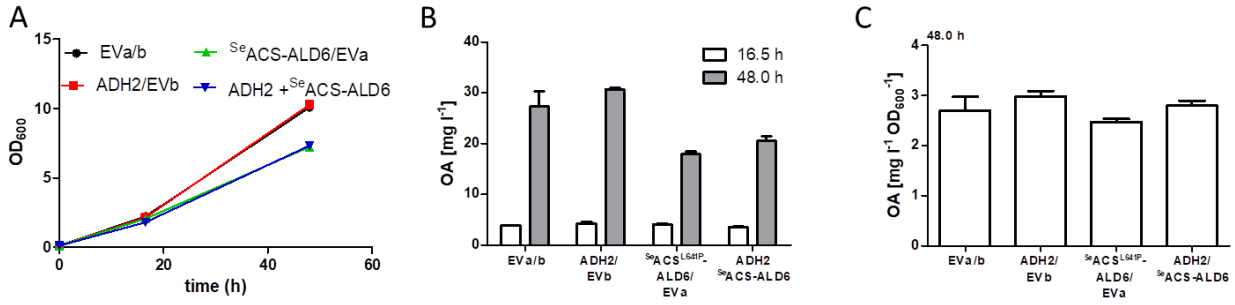
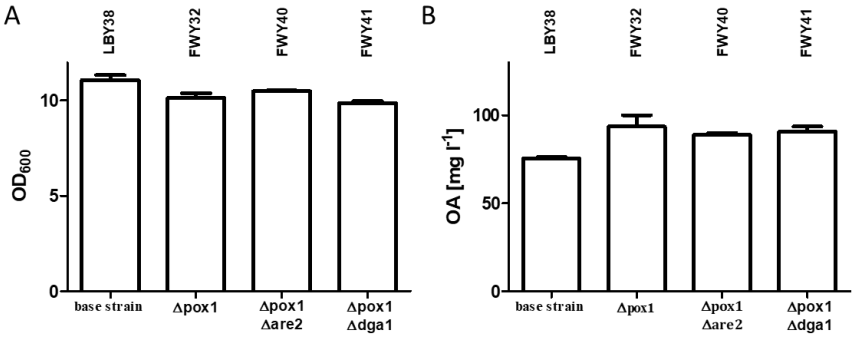


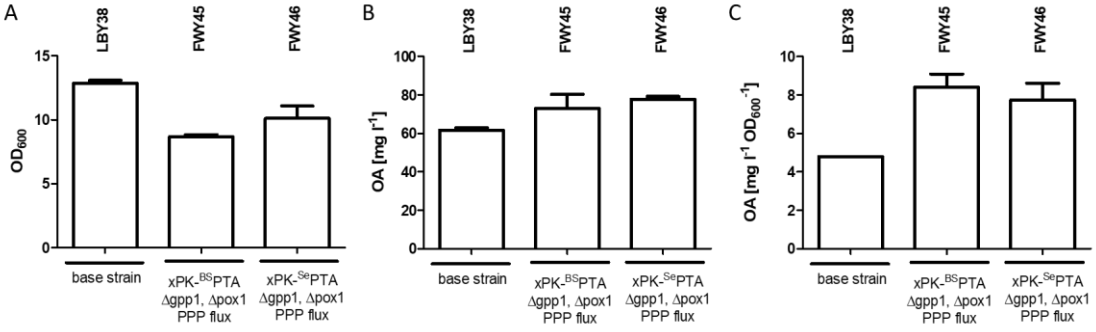
Supplementary Figure S1: Octanoic acid production of strains VGY2 and LBY38. Strains were grown in phosphate buffered SCD medium and growth (A) and octanoic acid production (B) was monitored over 72 h. Samples represent mean and standard deviation of two biological replicates. Error bars may be smaller than symbols.



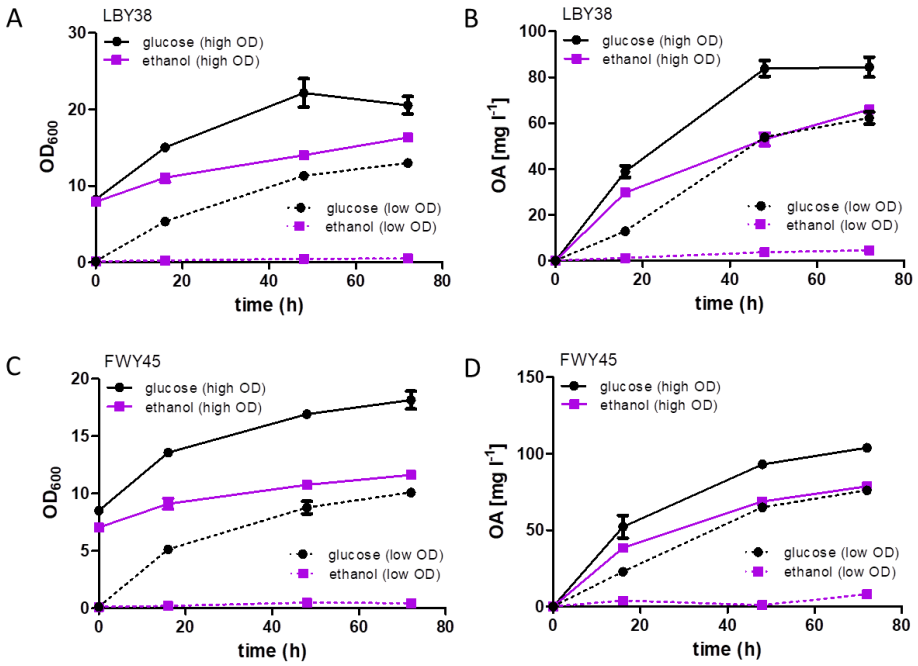
Supplementary Figure S2: Overexpression of PDH-bypass genes for octanoic acid biosynthesis. Strain LBY38 expressing high copy plasmids TWRV1 (*pHXT7*$^{1-392}$ *-ADH2*) or FWV171 (*pPGI1-ALD6, pPFK1-*^{Se}*ACS*^{L641P}) was grown in potassium phosphate buffered YPD media for 48 h with appropriate antibiotics for plasmid selection. Two appropriate high copy empty vectors (EVa and b) were used as control. (A) cell growth, (B) OA concentrations and (C) specific titers were analyzed. Values and error bars represent mean and standard deviation of two biological replicates.



Supplementary Figure S3: Deletion of β-oxidation and storage lipid formation in octanoic acid producing strians. (A) Growth and (B) octanoic acid production of strain with deletion in genes relevant for β-oxidation (*POX1*) and storage lipid formation (*ARE2*, *DGA1*). Samples were taken after 48 h of fermentation. Values represent mean and standard deviation of two biological replicates.



Supplementary Figure S4: Comparison of two different PTAs. (A) Growth, (B) OA production and (C) specific titers of a control strain (LBY38) or strains either expressing a PTA from *Bacillus subtilis* (^{BS}PTA) or *Salmonella enterica* (^{Se}PTA). Samples were taken after 48 h. Values show mean and standard deviation of two biological replicates.



Supplementary Figure S5: Octanoic acid production from glucose or ethanol in high OD fermentations compared to low OD fermentations. Strains LBY38 (A and B) or FWY45 (C and D) were inoculated to an OD of 0.1 (low OD, dashed lines) or 8.0 (high OD, solid lines) in potassium buffered YPD media containing either 0.1 mol/L (20.0 g l^{-1}glucose (black) or 0.2 mol l^{-1} (9.3 g l^{-1}) ethanol (violet). Samples show mean and standard deviation of two biological replicates.

References

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