SUPPLEMENTARY MATERIALS

Advances in Lycopene Production: From Natural Sources to Microbial Synthesis Using *Yarrowia lipolytica*

Authors: Paweł Moroz [1], Aleksandra Bartusiak [1], Julia Niewiadomska [1], Kacper Szymański [1], Tomasz Janek [1], Anna Kancelista [1], Anna Gliszczyńska [2] and Zbigniew Lazar [1]

1- Departament of Biotechnology and Food Microbiology, Wroclaw University of Environmental and Life Sciences, Chelmonskiego 37, 51-630, Wroclaw, Poland
2- Department of Food Chemistry and Biocatalysis, Wroclaw University of Environmental and Life Sciences, Norwida 25, 50-375 Wroclaw, Poland

1. Synthetic genes

Sequences of the synthetic genes were ordered from GeneArt (Thermo Fisher Scientific) and were codon optimized for *Y. lipolytica* using algorithm available on Benchling platform (http://benchling.com/). All genes come from *Pantoea agglomerans.*

Sequences used for construction of Golden Gate plasmids

GGA-PS (*phytoene synthase - crtB)*

gcatCGTCTCATCGGTCTCAAATGTCCCAGCCTCCCTTGCTAGACCACGCCACTCAG
ACCATGGCAAACGGATCTAAATCTTTTGCCACGGCCGCTAAGTTGTTCGACCCCGC
TACCAGGAGATCGGTCCTGATGCTTTACACCTGGTGTCGACACTGTGACGACGTCA
TTGACGACCAGACCCATGGCTTCGCTTCCGAGGCCGCCGCTGAGGAGGAGGCTAC
CCAACGACTGGCCCGACTCCGTACCCTAACTCTGGCGGCTTTCGAGGGCGCGGAG
ATGCAGGACCCCGCCTTCGCGGCCTTTCAGGAAGTTGCACTTACCCACGGGATTAC
GCCTCGAATGGCTCTCGACCATCTGGATGGATTCGCCATGGACGTCGCTCAAACAC
GATACGTGACATTTGAAGATACTCTACGCTACTGCTATCACGTTGCCGGTGTGGTTG
GTCTGATGATGGCCAGAGTGATGGGTGTGCGAGATGAACGAGTGCTGGACAGAGC
CTGCGACCTTGGACTTGCCTTCCAGCTCACGAATATCGCTCGGGACATCATTGACG
ACGCCGCTATTGATCGTTGCTACCTGCCCGCTGAGTGGTTGCAAGATGCGGGCTTG
ACACCCGAGAACTACGCTGCCCGAGAAAACAGAGCTGCGCTCGCTCGAGTCGCT
GAGCGGCTGATTGACGCCGCTGAACCATACTACATTTCCAGTCAGGCTGGCCTACA
TGATTTGCCCCCCAGATGCGCCTGGGCCATTGCAACAGCGCGGAGTGTTTACCGAG
AAATCGGTATCAAGGTCAAAGCCGCTGGTGGATCGGCCTGGGACCGTAGACAGCA
CACCTCTAAGGGCGAAAAAATCGCCATGCTGATGGCCGCGCCGGGACAGGTCATT
CGGGCCAAGACAACAAGGGTCACGCCACGACCAGCCGGGCTTTGGCAGCGACCA
GTCTAATCTATGAGACCTGAGACGgcat

GGA-PD (*phytoene desaturase - crtI)*

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TTCGACGCCGGACCCACGGTTATCACTGACCCTACTGCGCTGGAGGCTCTTTTCAC
ACTCGCTGGCCGGCGCATGGAAGATTACGTGAGACTGCTGCCTGTAAAGCCATTCT
ACCGACTGTGTTGGGAGTCTGGAAAGACTCTGGACTATGCCAACGATTCTGCTGA
GCTTGAAGCTCAAATCACTCAGTTTAACCCCAGGGATGTCGAGGGATACCGACGG
TTCCTTGCCTACTCTCAAGCAGTGTTCCAAGAGGGGTACCTGCGTCTGGGATCTGT
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CGACAGGCATTTTCTTTCCACTCCCTTCTGGTGGGAGGAAACCCCTTCACTACTTC
CTCTATCTACACTCTAATCCACGCCCTGGAGCGAGAATGGGGCGTGTGGTTTCCCG
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CGGCGAGATAGAGCTTAATGCGCGAGTTGAAGAGCTGGTGGTAGCAGACAATAGA
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GGTGTGCCTCGTTCTATGTGCTCGCCCCTGTGCCTCATCTGGGTAACGCGCCTCTG
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AGCGATATATGCCCGGTCTGCGTTCTCAGCTGGTCACTCAACGAATCTTTACACCG
GCCGACTTCCACGACACCCTGGACGCTCACCTTGGATCGGCTTTCTCTATTGAACC
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ACCTGTATCTCGTCGGAGCTGGCACACATCCGGGAGCCGGCATTCCGGGAGTGGT
CGCTTCTGCAAAGGCTACTGCCTCGCTGATGATCGAGGACCTGCAATGATCTATGA
GACCTGAGACGgcat

GGA-GGDS (*geranylgeranyl diphosphate synthase - crtE)*

gcatCGTCTCATCGGTCTCAAATGGTGTCTGGATCTAAGGCAGGGGTGAGTCCTCAC
CGGGAGATTGAGGTGATGCGTCAGTCAATCGACGACCATCTGGCGGGGCTGCTGC
CCGAAACCGACAGCCAGGACATCGTGTCCCTGGCCATGCGAGAGGGCGTGATGGC
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CCCGGAGACTGGCAAAGATAGAAACAAGGATGCCGGTAAGTCTACCCTTGTTAAC
CGACTCGGCGCCGACGCGGCTCGACAGAAGCTGCGCGAGCACATCGACAGCGCA
GACAAGCATCTGACGTTCGCTTGTCCACAGGGAGGTGCTATTCGACAGTTCATGCA
CCTGTGGTTTGGCCACCACTTGGCTGATTGGAGCCCTGTCATGAAGATTGCCTAGT
CTATGAGACCTGAGACGgcat

GGA-IDI (*isopentenyl diphosphate isomerase - idi)*

gcatCGTCTCATCGGTCTCAAATGAAGGACGAGAGACTGGTCCAGCGAAAGAACGA
TCACCTTGATATCGTTTTGGACCCGCGCCGTGCTGTTACTCAGGCCTCCGCGGGATT
TGAGCGATGGCGTTTTACGCACTGTGCTCTCCCGGAGTTGAACTTCTCAGATATCA
CGCTTGAAACGACCTTTTTGAACCGACAGCTTCAGGCCCCGCTGCTAATTTCTTCC
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CGACGCCGGACTCGGCCTCGATAAGACCCTGCGGCAGCTCGCCCCAGACGTCCCA
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TTGCAGGAGGCCCTGCAGCCTGGAGGCGACAGAGACTGGCGTGGCCGACTGGCC
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CCGCCACAGAACAACAACGAAGCGTGGCTAACGTGTTCGCGGACTGGGGCATTCC
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CTAGCGGTGGTATTAAGAACGGCGTGGACGCGGCAAAGGCCCTCCGACTGGGCGC
CTGCATGGTGGGACAGGCTGCTGCTGTCCTGGGCAGTGCGGGTGTGTCTACAGAG
AAAGTTATTGACCACTTTAACGTGATCATTGAGCAATTGCGAGTTGCATGCTTCTGT
ACTGGCTCCAGATCTCTTTCGGATCTTAAGCAAGCTGACATTCGATACGTTCGGGA
CACACCCTGATCTATGAGACCTGAGACGgcat

Sequences used for Gibson Assembly construction of the modified replicative vector

pHR-PS (*phytoene synthase;* WP_312633718.1*)* – TEF promoter – PS gene – XPR2 terminator

AGAGACCGGGTTGGCGGCGTATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAA
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CCACATATCAAACCTCCCCCGGTTCCCACACTTGCCGTTAAGGGCGTAGGGTACTG
CAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCG
GGTAACCCATGCCGGACGCAAAATAGACTACTGAAAATTTTTTTGCTTTGTGGTTG
GGACTTTAGCCAAGGGTATAAAAGACCACCGTCCCCGAATTACCTTTCCTCTTCTTT

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TGGCCGGCGCATGGAAGATTACGTGAGACTGCTGCCTGTAAAGCCATTCTACCGAC
TGTGTTGGGAGTCTGGAAAGACTCTGGACTATGCCAACGATTCTGCTGAGCTTGA
AGCTCAAATCACTCAGTTTAACCCCAGGGATGTCGAGGGATACCGACGGTTCCTTG
CCTACTCTCAAGCAGTCTTCCAAGAGGGGTACCTGCGTCTGGGATCTGTGCCCTTT
CTTTCTTTTCGAGACATGCTTAGAGCTGGGCCCCAGCTCTTGAAGCTGCAGGCATG
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GGACTGAACCAGCCCCATTCCCAGCTAGCTCATCACACCATCTGCTTTGGCCCGCG
GTACCGGGAGCTCATTGATGAGATCTTCACCGGGTCGGCTCTAGCCGACGATTTCT
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CCTCGTTCTATGTGCTCGCCCCTGTGCCTCATCTGGGTAACGCGCCTCTGGATTGGG
CCCAGGAGGGTCCCAAGCTCCGTGACCGTATTTTTGACTACCTGGAGGAGCGATAT
ATGCCCGGTCTGCGTTCTCAGCTGGTCACTCAACGAATCTTTACACCGGCCGACTT
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AGCTATTTATCACTCTTTACAACTTCTACCTCAACTATCTACTTTAATAAATGAATATC
GTTTATTCTCTATGATTACTGTATATGCGTTCCTCTAAGACAAATCG

pHR-PD (*phytoene desaturase)* TEF promoter – PD gene – XPR2 terminator

AGAGACCGGGTTGGCGGCGTATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAA
TTGACCCCAAATTGACCCAGTAGCGGGCCCAACCCCGGCGAGAGCCCCCTTCACC
CCACATATCAAACCTCCCCCGGTTCCCACACTTGCCGTTAAGGGCGTAGGGTACTG
CAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCG
GGTAACCCATGCCGGACGCAAAATAGACTACTGAAAATTTTTTTGCTTTGTGGTTG
GGACTTTAGCCAAGGGTATAAAAGACCACCGTCCCCGAATTACCTTTCCTCTTCTTT
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AGAATCATTCAAAATGTCCCAGCCTCCCTTGCTAGACCACGCCACTCAGACCATGG
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AGATCGGTCCTGATGCTTTACACCTGGTGTCGACACTGTGACGACGTCATTGACGA
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CTGGCCCGACTCCGTACCCTAACTCTGGCGGCTTTCGAGGGCGCGGAGATGCAGG
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GACATTCGAAGACACTCTACGCTACTGCTATCACGTTGCCGGTGTGGTTGGTCTGA
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GCCCCCCAGATGCGCCTGGGCCATTGCAACAGCGCGGAGTGTTTACCGAGAAATC
GGTATCAAGGTCAAAGCCGCTGGTGGATCGGCCTGGGACCGTAGACAGCACACCT
CTAAGGGCGAAAAAATCGCCATGCTGATGGCCGCGCCGGGACAGGTCATTCGGGC
GAAGACAACAAGGGTCACGCCACGACCAGCCGGGCTTTGGCAGAGACCAGTCTA
GGTGTCTGTGGTATCTAAGCTATTTATCACTCTTTACAACTTCTACCTCAACTATCTA
CTTTAATAAATGAATATCGTTTATTCTCTATGATTACTGTATATGCGTTCCTCTAAGAC
AAATCG

pHR-GGDS (*geranylgeranyl diphosphate synthase - crtE)* – TEF promoter – GGDS gene – LIP2 terminator

AGAGACCGGGTTGGCGGCGTATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAA
TTGACCCCAAATTGACCCAGTAGCGGGCCCAACCCCGGCGAGAGCCCCCTTCACC
CCACATATCAAACCTCCCCCGGTTCCCACACTTGCCGTTAAGGGCGTAGGGTACTG
CAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCG
GGTAACCCATGCCGGACGCAAAATAGACTACTGAAAATTTTTTTGCTTTGTGGTTG
GGACTTTAGCCAAGGGTATAAAAGACCACCGTCCCCGAATTACCTTTCCTCTTCTTT
TCTCTCTCTCCTTGTCAACTCACACCCGAAATCGTTAAGCATTTCCTTCTGAGTATA
AGAATCATTCAAAGATGGTCTCTGGATCTAAGGCAGGGGTGAGTCCTCACCGGGA
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CCTTGACTTTGGTCAGGCCTTCCAGCTCCTCGACGACCTGCGGGATGATCACCCGG
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GCATCTGACGTTCGCTTGTCCACAGGGAGGTGCTATTCGACAGTTCATGCACCTGT
GGTTTGGCCACCACTTGGCTGATTGGAGCCCTGTCATGAAGATTGCCTGAGTGTCT

GTGGTATCTAAGCTATTTATCACTCTTTACAACTTCTACCTCAACTATCTACTTTAAT
AAATGAATATCGTTTATTCTCTATGATTACTGTATATGCGTTCCTCTAAGACAAATCG

pHR-IDI (*isopentenyl diphosphate isomerase - idi)* – TEF promoter – IDI gene – LIP2 terminator

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TTGACCCCAAATTGACCCAGTAGCGGGCCCAACCCCGGCGAGAGCCCCCTTCACC
CCACATATCAAACCTCCCCCGGTTCCCACACTTGCCGTTAAGGGCGTAGGGTACTG
CAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCG
GGTAACCCATGCCGGACGCAAAATAGACTACTGAAAATTTTTTTGCTTTGTGGTTG
GGACTTTAGCCAAGGGTATAAAAGACCACCGTCCCCGAATTACCTTTCCTCTTCTTT
TCTCTCTCTCCTTGTCAACTCACACCCGAAATCGTTAAGCATTTCCTTCTGAGTATA
AGAATCATTCAAAATGAAGGACGAGAGACTGGTCCAGCGAAAGAACGATCACCTT
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ATGGCGTTTTACGCACTGTGCTCTCCCGGAGTTGAACTTCTCAGATATCACGCTTG
AAACGACCTTTTTGAACCGACAGCTTCAGGCCCCGCTGCTAATTTCTTCCATGACT
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TGCCGGAGCCGGTGGCACCTCATGGGCCGCCGTAGAGGGTGAGCGAGCCGCCAC
AGAACAACAACGAAGCGTGGCTAACGTGTTCGCGGACTGGGGCATTCCTACAGCC
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TGAGTGTCTGTGGTATCTAAGCTATTTATCACTCTTTACAACTTCTACCTCAACTATC
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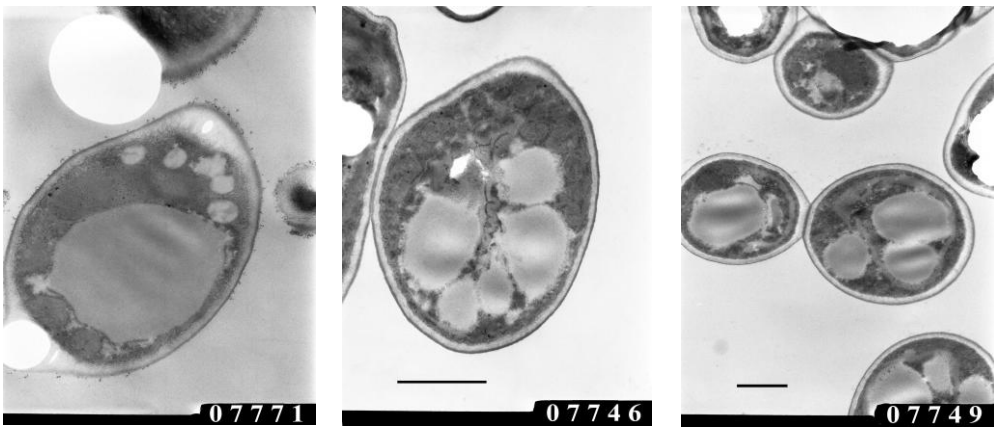
2. Primers used in this experiment

The primers listed in Table S1 were used to amplify the genes for construction of JMP62 type vectors with single genes overexpression as well as for transformants verification and RT-qPCR.

Table S1. List of primers used in this study.

Name of primer	Sequence
PD-BamHI-F	atcgGGATCCATGAAGAAAACAGTCGTAATCGGCG
PD-AvrII-R	gctaCCTAGGTCATTGCAGGTCCTCGATCATCAG
PS-BamHI-F	gatcGGATCCATGTCCCAGCCTCCCTTGCTAG
PS-AvrII-R	gatcCCTAGGTTAGACTGGTCGCTGCCAAAGC
GGDS-BamHI-F	gctaGGATCCATGGTGTCTGGATCTAAGGCAGGG
GGDS-AvrII-R	tagcCCTAGGCTAGGCAATCTTCATGACAGGGC
IDI-BamHI-F	actgGGATCCATGAAGGACGAGAGACTGGTCCAG
IDI-AvrII-R	tagcCCTAGGTCAGGGTGTGTCCCGAACG
TEF-F	CTCCTTGTCAACTCACACCCG
LIP2-R	CTTAGAGGAACGCATATACAGTAATCATAGAG
RT-qPCR	
crtI-rev	AACACTGCTTGAGAGTAGGCAA
crtI-fwd	CTGTGTTGGGAGTCTGGAAAGA
crtB-rev	CAGGTAGCAACGATCAATAGCG
crtB-fwd	GTCTGATGATGGCCAGAGTGAT
crtE-rev	TTCTATCTTTGCCAGTCTCCGG
crtE-fwd	CTACAGATTGTCGCTATCGCCT
IDI-rev	CTCCCATGGCAATCTTCAACAC
IDI-fwd	AACGACCTTTTTGAACCGACAG
Act-fwd	CTGGGATGACATGGAGAAGATCTGGC
Act-rev	GAGGCGTACAGGGAGAGGACG

3. Electron microscopy picture of *Y. lipolytica* wild type (W29) and phospholipid overproducing (PS05) strains growing in YNB glucose media.

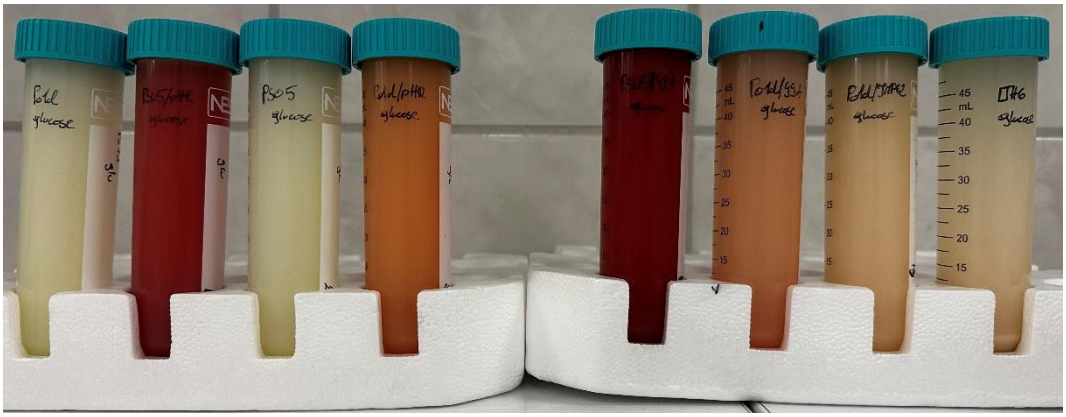


W29 **PS05** **PS05**

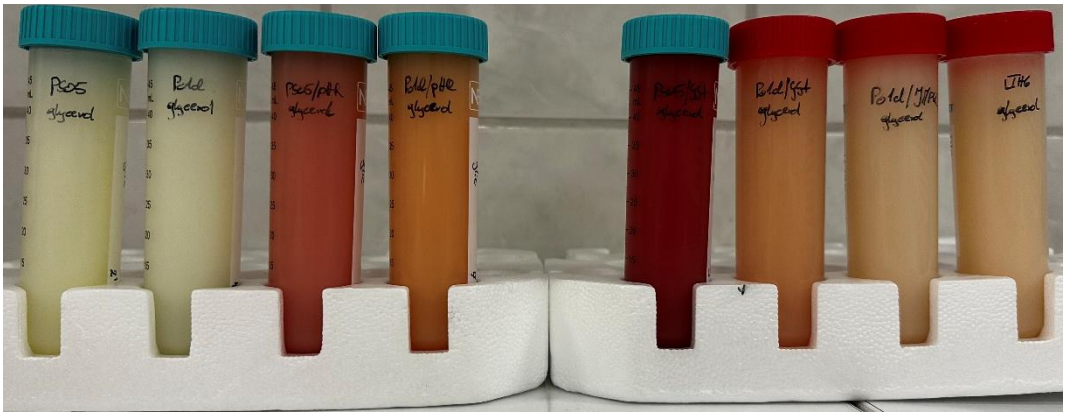
Figure S1. Morphology of *Y. lipolytica* strains (W29 – wild type, PS05 – phospholipid overproducing strain) growing in a YNB medium with glucose.

4. Carbon source-dependent colour differences in *Y. lipolytica* lycopene cultures depending on strain genotype and pathway integration method

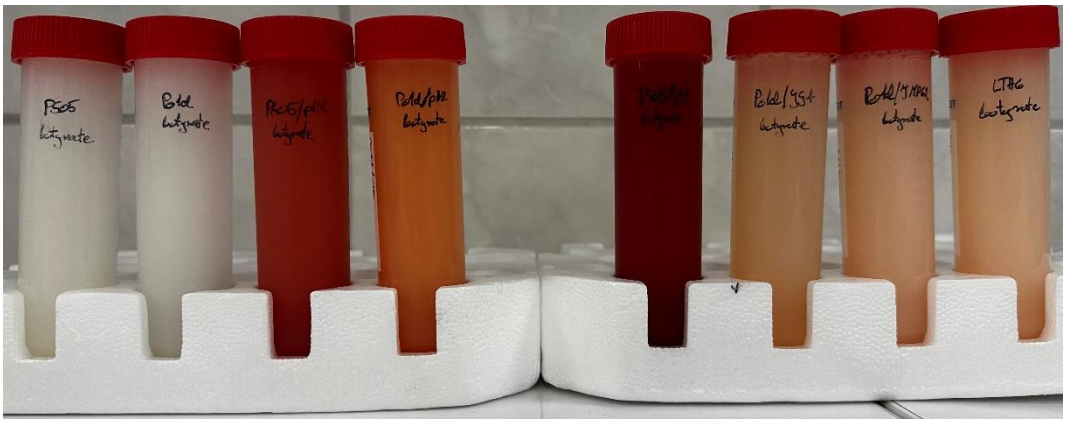
GLUCOSE



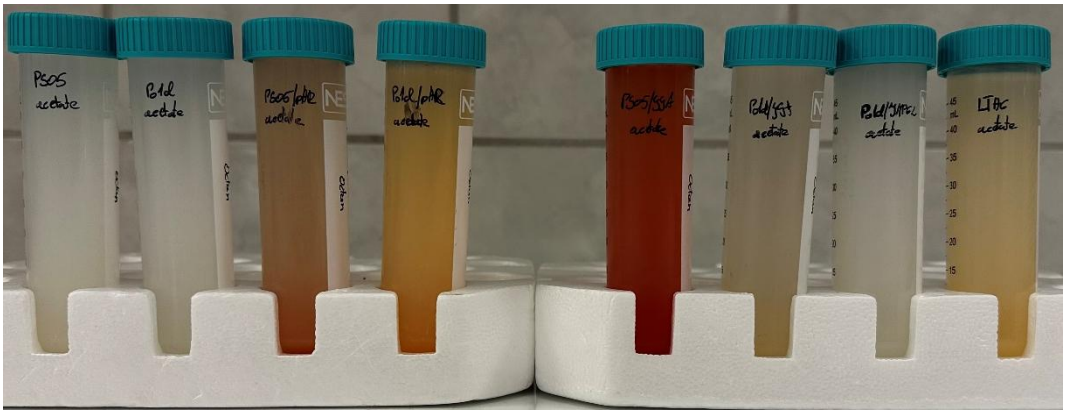
GLYCEROL



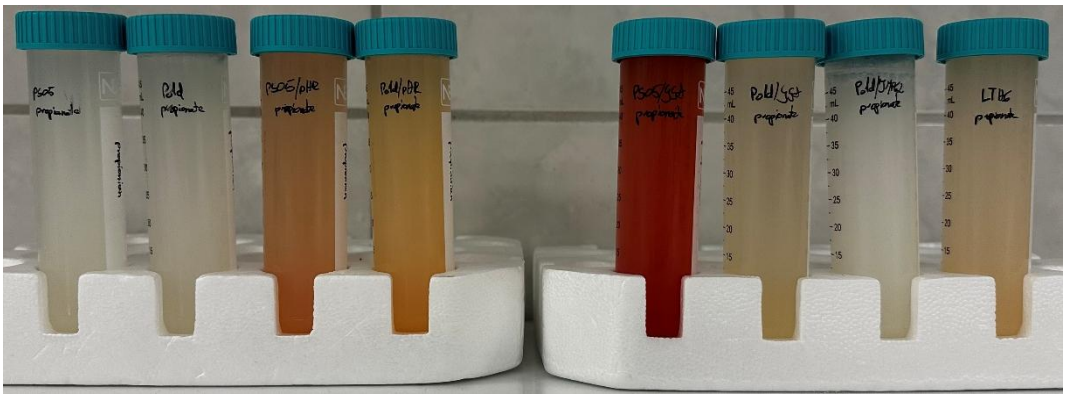
BUTYRATE



ACETATE



PROPIONATE



MIX (SCFA)

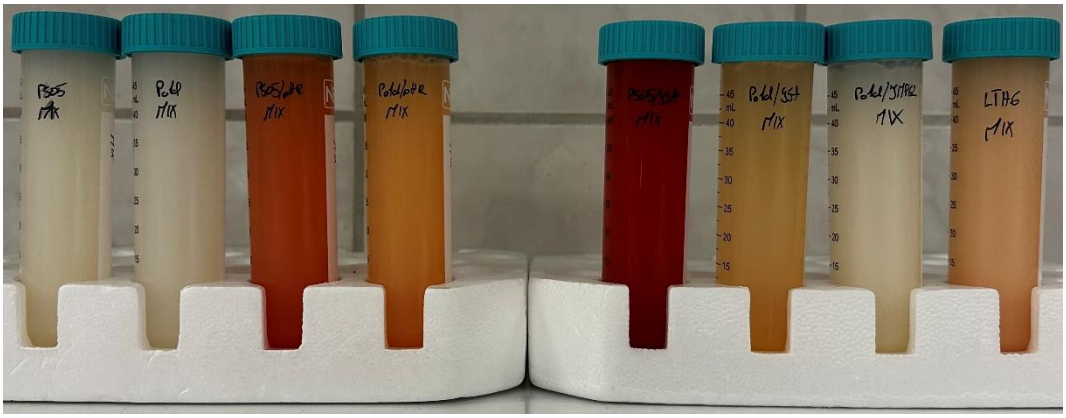
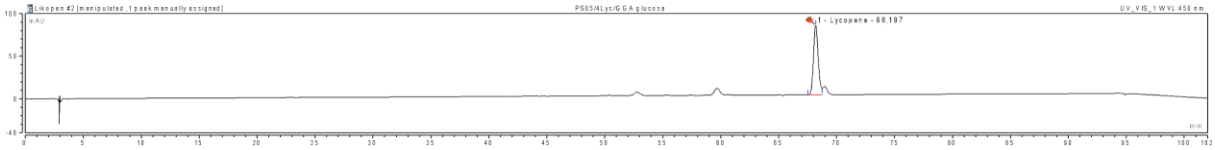


Figure S2. Effect of carbon source and genetic constructs on pigmentation and it's intensity in lycopene-producing *Y. lipolytica* transformants (order: PS05, PO1d, PS05/4lyc/pHR, PO1d/4lyc/pHR, PS05/4lyc/GGA, PO1d/4lyc/GGA, PO1d/JMP62, LTH6).

5. **Representative chromatogram of lycopene extraction from *Y. lipolytica* transformants, exemplified by strain PS05/4lyc/GGA.**

A)



B)

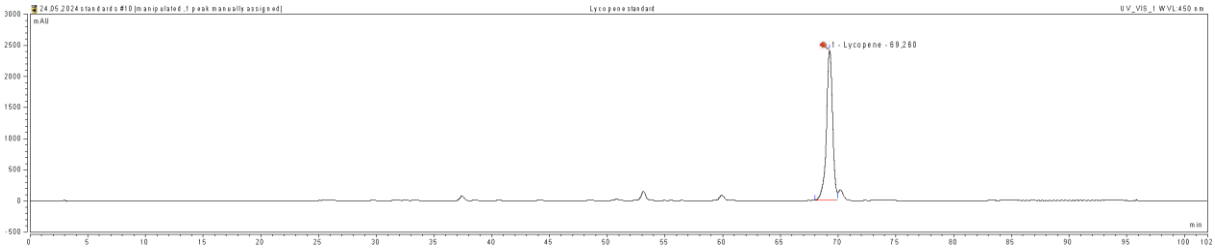


Figure S3. HPLC chromatographic profiles of lycopene. (A) Extract from *Y. lipolytica* strain PS05/4lyc/GGA. (B) Reference chromatogram of lycopene standard used for identification.