

**Engineering of a probiotic yeast for the production and secretion of medium-chain fatty acids
antagonistic to an opportunistic pathogen *Candida albicans***

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Supplementary Information

Table S1. Oligos used in this study

Table S2. DNA sequences used for MCFA biosynthesis

Figure S1. Functional analyses of commercial MCFAs against biofilm and hyphal formation in *C. albicans* SC5314

Figure S2. Hyphal formation of *C. albicans* 5314 treated by the CLPY04 supernatant and CLPY01 supernatant under different pH conditions

Figure S3. Growth patterns of CLPY04 and CLPY02 in YGD medium containing uracil

42 Table S1. Oligos used in this study
43

Name	Sequence (5'→3')
<i>Boulardii strain characterization</i>	
EI1	CTGGCTTGGTGTATGT
LA1	GCGATCGGTGTACTAAC
MatF	GAGAAGAGCCCAAAGGGAAAATCAT
Mat α F	GTCTAGTATGCTGGATTTAAACTCA
MatR	AGTCACATCAAGATCGTTTATGG
<i>Gene deletion and integration</i>	
pUG72-UraF	TATGGGAGGAAGAGAAGAAG
HygBR	GCAGCGATCGCATCCATTG
URA3uF	TAGAACGCGGCCGCGAACAAACACCAGAGTCAAA
URA3uR	TTTGGGACGCTCGAAGATGATTTATCTTCGTTTCCTGC
URA3dF	AGATCCACTAGTTGCATGTATACTAAACTCACAA
URA3dR	ATGCAGGTAAACGTTTTGTTCTTGGAACGCT
HR1F	GCAGGAAACGAAGATAAATCATCTTCGAGCGTCCCAAAA
HR1R	GTCGACCTGCAGGGTGGTTGAGTATTTGGTCT
HR2F	TAGAACGCGGCCGCTTGTTGCTGTTTTATTGACCA
HR2R	GTCGACCTGCAGTCTTCATCAGCCAATATACCC
HR3F	TAGAACGCGGCCGCAACATGCCTTCCTTGAAGAT
HR3R	GTCGACCTGCAGGAGCGACCTCATGCTATAC
Tef1p-Tdh3pF1	AATTAAGAGCTCAGTTTATCATTATCAATACT
Sfp-Tdh3pR1	GTCCATGTAGATACCGTATATCTTCATTTTGTGTTTATGT GTGTTT
Tdh3p-SfpF1	AAACACACATAAAACAAACAAAATGAAGATATACGGTATCT ACATGGAC
Cyc1t-SfpR2	CTAACTCCTTTCCTTTTCGGTTAGAGCGGATCTTAGCTAGCC GCGGTACCA
Tdh3p-Tef1pF2	AGATCTGAGCTCATAGCTTCAAAAATGTTTCTA
hFAS-rTE-Tef1pR2	ACGATAAGATCTTTCCATTTTGTAAATTAATACTTAGATTA
<i>RT-PCR</i>	
CaBCR1-F	AATAAACTGGATCCTCCACC
CaBCR1-R	GTAGCATTAGTAGTAGCCGT
CaEFG1-F	ATTACACAAGTGCTCCTAGT
CaEFG1-R	GGATAGGTACTGCTTGTTGA
qCaHGC1-F	AATGGTAGTGTTATGAGTGGA
qCaHGC1-R	GGAGAATCATTTTCACTAATAGGT
CaHWP1-F	CACAGGTAGACGGTCAAGGT
CaHWP1-R	AAGGTTCTTCCTGCTGTTGT
CaUME6-F	TTACACCCGATTCAACTTCT
CaUME6-R	GTA CTGGTGAAGACGA ACTA
CaACT1-F	TTTCATCTTCTGTATCAGAGGAACTTATTT
CaACT1-R	ATGGGATGAATCATCAAACAAGAG
IL-6-F	CTATGGAAGGGTCC TACTCA
IL-6-R	GGCTATGGGATGATGTTCAA
IL-8-F	TGCGCCAACACAGAAATTAT
IL-8-R	CTGTAATCCTAACACCTGGA
LL-37-F	AGAGAAGATTGGCAAAGAGTT
LL-37-R	GGGTACAAGATTCCGCAAAA
SA1009-F	CAGTTTGGGTTGACAAGGGA

SA1009-R	ATCTTCCCCTTGATTGCTGTA
CCL20-F	TCTCCTCAGGTATGTTACACT
CCL20-R	ACTCCTCTTTCCTCAAACATT
HBD3-F	GAGTCAGTATTTCTCCATCCT
HBD3-R	AGTTCCTATACAACAAACCT
ACTB-F	CTGGAACGGTGAAGGTGACA
ACTB-R	AAGGGACTTCCTGTAACAATGCA

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Table S2. DNA sequences used for MCFA biosynthesis

Name	Nucleotide sequence
hSFP	<u>ATG</u>GTTTTCCCAGCTAAGAGATTCTGTTTGGTTCCATCTATGGAAGGT GTTAGATGGGCTTTTTCTTGTGGTACTTGGTTGCCATCAAGAGCTGAA TGGTTGTTGGCTGTTAGATCAATTCAACCTGAAGAAAAAGAAAGAAT CGGTCAATTCGTTTTCGCTAGAGATGCTAAAGCTGCTATGGCTGGTA GATTGATGATTAGAAAATTGGTCGCCGAAAAGTTGAACATCCCTTGG AATCATATCAGATTGCAAAGAACTGCTAAGGGTAAACCAGTTTTGGC TAAGGATTCTTCTAATCCATACCCAACTTCAACTTCAACATCTCCCA TCAAGGTGATTATGCTGTTTTGGCTGCTGAACCAGAATTGCAAGTTG GTATCGATATTATGAAGACCTCATTTCCAGGTAGAGGTTCCATTCCA GAATTTTTCCATATCATGAAGAGAAAGTTCACCAACAAAGAATGGGA AACCATCAGATCCTTTAAGGATGAATGGACCCAATTAGACATGTTCT ACAGAAACTGGGCCTTGAAAGAATCTTTTATCAAGGCTATTGGTGTC GGTTTGGGTTTTCGAATTACAAAGATTGGAATTTGATTGTCCCCATTG AACTTGGACATCGGTCAAGTTTACAAAGAAACCAGATTATTCTTGGA TGGTGAAGAAGAAAAAGAATGGGCCTTCGAAGAATCCAAGATCGAT GAACATCATTTGTTGCTGTTGCTTTGAGAAAACCAGATGGTTCAAG ACATCAAGATGTTCCATCTCAAGATGATTCTAAGCCAACCTCAAAGAC AATTCACCATCTTGAACCTTCAACGACTTGATGTCATCTGCTGTTCCAA TGACTCCAGAAGATCCATCTTTTTGGGATTGCTTTTGTTCACCGAAG AAATCCCAATCAGAAACGGTACAAAATCCCATCATCATCACCATCAC <u>TGA</u>
P _{TDH3} -P _{TEF1}	TTTGTGTTGTTTATGTGTGTTTATTCGAAACTAAGTTCTTGGTGTTTTAA AACTAAAAAAGACTAACTATAAAAGTAGAATTTAAGAAGTTTAA GAAATAGATTTACAGAATTACAATCAATACCTACCGTCTTTATATAC TTATTAGTCAAGTAGGGGAATAATTTTCAGGGAAGTGGTTTCAACCTT TTTTTTCAGCTTTTTTCCAAATCAGAGAGAGCAGAAGGTAATAGAAGG TGTAAGAAAATGAGATAGATACATGCGTGGGTCAATTGCCTTGTGTC ATCATTTACTCCAGGCAGGTTGCATCACTCCATTGAGGTTGTGCCCGT TTTTTGCCTGTTTGTGCCCTGTTCTCTGTAGTTGCGCTAAGAGAATG GACCTATGAACTGATGGTTGGTGAAGAAAACAATATTTTGGTGCTGG GATTCTTTTTTTTTCTGGATGCCAGCTTAAAAAGCGGGCTCCATTATA TTTAGTGGATGCCAGGAATAAACTGTTCAACCAGACACCTACGATGT TATATATTCTGTGTAACCCGCCCTATTTTGGGCATGTACGGGTTAC AGCAGAATTAAAAGGCTAATTTTTTGAATAAAAGTTAGGAAAAT CACTACTATTAATTATTTACGTATTCTTTGAAATGGCAGTATTGATAA TGATAAACTGAGCTCATAGCTTCAAATGTTTCTACTCCTTTTTTACT CTTCAGATTTTCTCGGACTCCGCGCATCGCCGTACCACTTCAAACA CCCAAGCACAGCATACTAAATTTCCCTCTTTCTTCCTCTAGGGTGTC GTTAATTACCCGTACTAAAGGTTTGGAAAAGAAAAAAGAGACCGCC TCGTTTCTTTTTCTTCGTCGAAAAAGGCAATAAAAAATTTTATCACGT TTCTTTTTCTTGAAAATTTTTTTTTTTGATTTTTTCTTTTCGATGACC TCCATTGATATTTAAGTTAATAAACGGTCTTCAATTTCTCAAGTTTC AGTTTCATTTTTCTTGTCTATTACAACTTTTTTACTTCTTGCTCATT AGAAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAA
mhFAS-rTEII	<u>ATG</u>GAAAGATCTGAAGTCGTTATCGCCGGTATGTCTGGTAAATTGCC AGAATCTGAAAACCTTGCAAGAATTTGGGACAACCTTGATCGGTGGTG TTGATATGGTTACTGATGATGATAGAAGATGGAAGGCTGGTTTGTAT

	GGTTTGCCAAGAAGATCCGGTAAATTGAAGGATTTGTCCAGATTCTGA TGCTTCCTTTTTTGGTGTTTCATCCAAAGCAAGCTCATACAATGGACCC ACAATTAAGATTGTTGTTGGAAGTTACCTACGAAGCCATAGTTGATG GTGGTATTAACCCAGATTCTTTGAGAGGTACTCATACTGGTGTTTGG GTTGGTGTTTCTGGTTCTGAACTTCTGAAGCCTTGTCAGAGATCCA GAACTTTGGTTGGTTACTCTATGGTTGGTTGTCAAAGAGCTATGAT GGCTAACAGATTGTCATTCTTCTTCGATTTCCGTGGTCCATCTATTGC TTAGATACTGCTTGTTCCCTCTTCTTTGATGGCCTTGCAAAATGCTTA CCAAGCTATTCACTTCTGGTCAATGTCCAGCTGCTATAGTTGGTGGTAT CAATGTTTTGTTGAAGCCAAACACCTCCGTCCAATTTTTGAGATTGGG TATGTTGTCTCCAGAAGGTACTTGTAAGGCTTTTGATACAGCTGGTA ATGGTTATTGCAGATCCGAAGGTGTTGTTGCTGTTTTATTGACCAAAA AGTCCTTGGCCAGAAGAGTTTACGCTACTATTTTGAACGCTGGTACT AATACCGACGGTTTCAAAGAACAAGGTGTTACTTTTCCATCCGGTGA CATCCAAGAACAATTAATCAGATCCTTGTACCAATCTGCTGGTGTG CTCCTGAATCCTTCGAATATATTGAAGCTCATGGTACTGGTACAAAG GTTGGTGATCCACAAGAATTGAACGGTATTACTAGAGCTTTGTGTGC TACTAGACAAGAACCTTTGTTGATTGGTTCTACCAAGTCTAATATGG GTCATCCAGAACCAGCTTCAGGTTTGGCTGCTTTGGCTAAAGTTTTGT TGTCTTTGGAACATGGTTTGTGGGCTCCAAACTTGCATTTTCATTCTC CAAATCCAGAAATCCCAGCTTTGTTGGATGGTAGATTGCAAGTTGTT GACCAACCATTGCCAGTTAGAGGTGGTAATGTTGGTATCAACTCTTT TGGTTTTGGTGGTTCCAACGTCCACATTATCTTAAGACCAAATACTCA ACCACCACCAGCTCCAGCTCCACATGCTACTTTACCAAGATTATTGA GAGCTTCTGGTAGAACTCCAGAAGCAGTTCAAAAGTTATTGGAACAA GGTTTAAGACACTCCCAAGATTTGGCCTTTTTGTCCATGTTGAATGAT ATTGCTGCTGTTCCAGCTACTGCTATGCCTTTTAGAGGTTATGCTGTT TTGGGTGGTGAAAGAGGTGGTCCAGAAGTTCAACAAGTTCCAGCCG GTGAAAGACCATTGTGGTTTATTTGTTCTGGTATGGGTACTCAATGG AGAGGTATGGGTTTGTCTTTGATGAGATTGGATAGATTCAGAGACTC CATCTTGAGATCAGATGAAGCTGTAAAGCCATTCGGTTTGAAGGTCA GTCAATTATTGTTGTCCACTGACGAATCTACCTTCGATGATATTGTCC ACTCTTTCGTTTCTTGACCGCCATTCAAATTGGTTTGATCGATTTGTT GTCCTGCATGGGTTTAAGACCAGATGGTATAGTTGGTCATTCTTTGG GTGAAGTTGCTTGTGGTTACGCAGATGGTTGTTTGTCTCAAGAAGAA GCTGTTTTAGCTGCTTATTGGAGAGGTCAATGTATCAAAGAAGCACA TTTGCCACCAGGTGCTATGGCTGCTGTTGGTTTATCTTGGGAAGAATG TAAACAAAGATGTCCACCTGGTGTGTTCCAGCATGTCATAATTCTA AGGATACCGTTACTATCTCTGGTCCACAAGCTCCAGTTTTTCGAATTTG TCGAACAATTAAGAAAAGAAGGTGTTTTCGCCAAAGAAGTTAGAAC TGGTGGTATGGCTTTCCACTCTTACTTTATGGAAGCTATTGCTCCACC ATTATTGCAAGAATTGAAGAAGGTTATCAGAGAACCTAAGCCAAGA TCAGCTAGATGGTTATCTACTTCTATTCCTGAAGCTCAATGGCATTCT AGTTTGGCTAGAACTTCTTCTGCTGAATACAACGTTAACAACCTGGTT TCCCCAGTTTTGTTCCAAGAAGCCTTATGGCATGTTCCAGAACACGCT GTTGTTTTAGAAATTGCTCCTCATGCTTTGTTGCAAGCCGTTTTGAAA AGAGGTTTGAAGCCATCCTGTACCATTATCCCATTGATGAAGAAGGA TCACAGAGACAACCTTGGAATTTTTCTTGGCTGGTATCGGTAGATTAC ACTTGTCTGGTATTGATGCTAATCCAAACGCTTTGTTTCCACCAGTTG AATTTCCAGCTCCTAGAGGTACACCATTGATTTACCTTTGATTAAGT GGGATCATTCTTGGCTTGGGATGTTCTGCTGCTGAAGATTTTCCAA
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	AAACTTCATTAAGAACTACATTGCCAAGTGCTTGGAATTGTCATCAT TGACTCATCATCACCATCACC <u>ACTGA</u>
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Figure S1

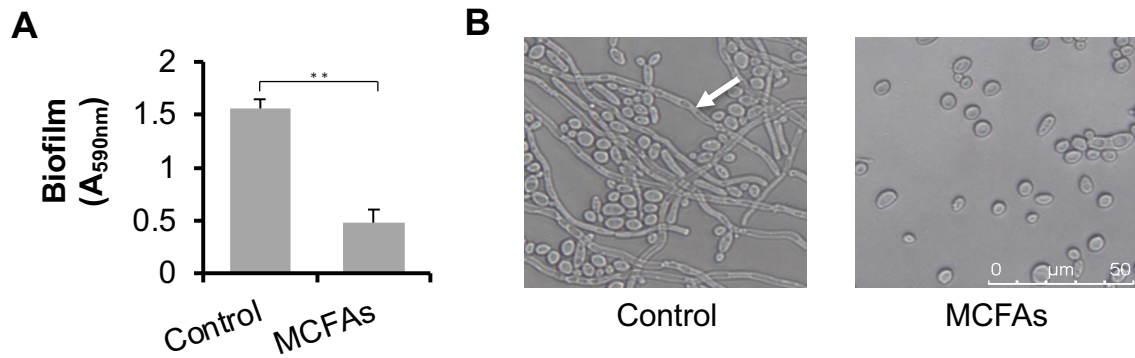


Figure S1. Functional analyses of the commercial MCFAs against biofilm and hyphal formation in *C. albicans* SC5314. A. Effect of the commercial MCFAs on biofilm formation. B. Effect of the commercial MCFA on hyphal formation. Control, without MCFAs. The commercial MCFAs were prepared by mixing respective MCFA (C6:0, C8:0, and C10:0) at the same concentrations as those in the supernatant of the MCFA-producing strains. Hypha is indicated by an arrow. **, $p < 0.01$ (Student's t-test).

Figure S2

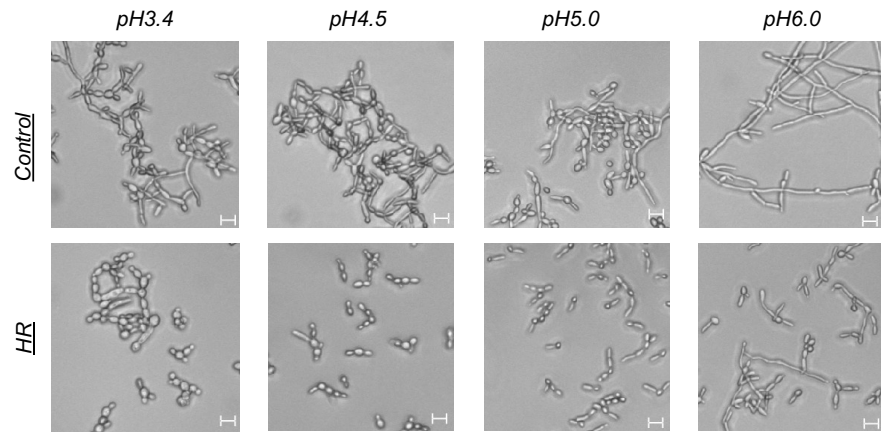


Figure S2. Hyphal formation of *C. albicans* 5314 treated by the CLPY04 supernatant (Supernatant HR) and CLPY01 supernatant (Supernatant C) under different pH conditions. Scale bar represents 5 μ m.

Figure S3

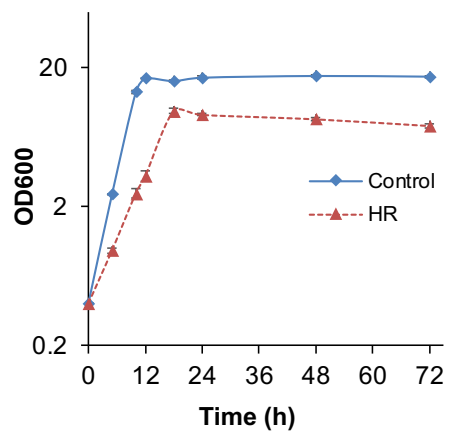


Figure S3. Growth patterns of CLPY04 and CLPY02 in YGD medium containing uracil.
Three independent experiments were performed. Control, CLPY02, HR, CLPY04.