Electronic Supplementary Material (ESI) for Sustainable Energy & Fuels.

Supporting information

Bacillus subtilis spore surface display of photodecarboxylase for the transformation of lipids to hydrocarbons

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Seq. S1: DNA sequence of cotY-linker-*Cv*FAP codon optimized for *B. subtilis* used in plasmids p02027, p02030 (cotY = bold, linker = underlined)

ATGAGCTGCGGAAAAACCCATGGCCGGCATGAGAACTGTGTATGCGATGCAGTGGAA
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TGCTTTGAATCCATTTTCTTCCGCGTCGAAAAATTATGCGATTGCTGTGCAACACTGTCT
ATTTTACGCCCGGTCGATGTCAAAGGCGATACCTTAAGTGTTTGCCACCCTTGCGACC
CGGATTTCTTCGGGCTAGAAAAAACAGATTTCTGCATTGAAGTGGATCTCGGATGCTTC
TGCGCGATTCAGTGCCTGTCACCAGAGCTAGTTGACAGAACATCGCCTCACAAAGATA
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GGCGGGCCAAAAATATGATTATATTCTGGTTGGCGGCGGCACAGCAGCATGCGTCCTGG
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ATAATACAAGCCGCGATGTTAAAATTCCGGCGGCAATTACAAGACTGTTTAGATCACCGC
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CTAGAGGCAGACTGTTAGGCGGCAGCAGCGCAACAAATGCCACACTGTATCATAGAGGA
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CTTGGTTTGTGCAAGCGGAAACAAATGCAGATTTTGGGCCTGGCGCGTATCATGGCAGC
GGTGGACCGATGCGAGTGGAAAACCCGAGATATACAAATAAACAACTGCATACAGCGTT
TTTCAAAGCAGCGGAAGAAGTAGGCTTAACACCGAACTCCGATTTCAACGACTGGTCAC
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CATTCATTGGGCAAGAGATGTGGCGAGAAGCAGCGCGCTTAGCGAATATCTTGATGGCG
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AAGCGTTGTCGATAATCAACTGAGAGTGCATGGCGTTGAAGGACTGAGAGTTGTTGATG
CAAGCGTTGTGCCGAAAATTCCGGGCGGCCAAACAGGCGCGCCGGTTGTTATGATTGC
GGAAAGAGCGGCAGCGCTTCTGACAGGCAAAGCAACAATTGGCGCGAGCGCAGCAGC
ACCGGCGACAGTTGCAGCACATCATCATCATCATCACTAA

Seq. S2: DNA sequence of cotY-linker-RO lipase codon optimized for *B. subtilis* used in plasmid p95002 (cotY = bold, linker = underlined)

ATGGTGCCAGTGAGCGGGAAATCAGGCAGTTCAAACACGGCTGTATCAGCGAGTGACA
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GAACTGTGTATGCGATGCAGTGGAAAAGATTTTAGCAGAGCAGGAGGCAGTTGAAGA
ACAGTGTCCGACTGGCTGCTATACCAACCTTTTAAACCCTACGATTGCTGGAAAAGAC

ACAATTCCGTTTCTCGTTTTTGATAAAAAAGGCGGATTGTTCTCCACATTCGGAAACGT AGGGGGATTTGTGGATGATATGCAATGCTTTGAATCCATTTTCTTCCGCGTCGAAAAAT TATGCGATTGCTGTGCAACACTGTCTATTTTACGCCCGGTCGATGTCAAAGGCGATACC TTAAGTGTTTGCCACCCTTGCGACCCGGATTTCTTCGGGCTAGAAAAAACAGATTTCTG CATTGAAGTGGATCTCGGATGCTTCTGCGCGATTCAGTGCCTGTCACCAGAGCTAGTT GACAGAACATCGCCTCACAAAGATAAAAAGCATCATCACAATGGATAA

Table S1. List of strains employed in the present study.

Strain	**Genotype**	**Resistance**	**Reference**
Bacillus subtilis KO7	*ΔnprE ΔaprE Δepr Δmpr ΔnprB Δvpr Δbpr*	no	*Bacillus Genetic Stock Center ID 1A1133*
Bs02003	*ΔsacA::(Zeo^R, P_{xylA}-cre, P_{spac}-comS, lacI), ΔcwlD::lox72, ΔsleB::lox72*	zeo^R	Previous study [1]
Bs02035	*ΔsacA::(Zeo^R, P_{xylA}-cre, P_{spac}-comS, lacI), ΔcwlD::lox72, ΔsleB::lox72 ΔpksX:: (P_{cotYZ}-cotY-linker-CvFAP)*	zeo^R	This work
Bs02039	Bs02035 + replicative plasmid p02030	Kan^R	This work
Bs95002	Bs02003 + replicative plasmid p95002	Kan^R	BSc thesis of Peter Gockel

Table S2. Plasmids employed in the present study

Plasmid	**Backbone**	**Insert**	**Integration site**	**Resistance**	**Reference**
p02010	pUC57	*CvFAP*	-	Amp	This study
p02027	pJK179	*PcotYZ-cotY-CvFAP*	*pksX*	spec	This study
p02030	p14037	ORI: colE1 (Ec), repE (Bs); KanR; Resolvase beta;PcotYZ,cotY, CvFAP	-	kan	This study
p95002	p14037	ORI: colE1 (Ec), repE (Bs); KanR; Resolvase beta;PcotYZ,cotY,ROL(*R. oryzae* lipase)	-	kan	BSc thesis of Peter Gockel

Table S3. Employed oligonucleotides (fw: forward, rv: reverse, bold: primer binding region)

Oligo name	Sequence (5'-3')	Description
02124_PcotYZ_pksx_OH_fw	TTATAAAAATTGCCCTCTCATT TTTTTCTTGTTAC**CCAAGCAT ATGATGAATATATAGACG**	amplification of *cotY* and its promoter from chromosome (Bs02003) with overhang to the *pksX* homology region
02174_cotY_OH_rv	ACTTCCACCACCTCCACCAC TTCCACCACCTCCACC**TCCAT TGTGATGATGCTTTTTATC**	amplification of *cotY* and its promoter from chromosome (Bs02003) with overhang to the linker
02175_cvFAP_OH_linker_fw	GGTGGAGGTGGTGGAAGTG GTGGAGGTGGTGGAAGT**ATG AGAGCGAGCGCAG**	amplification of *Cv*FAP from p02010 with overhang to the linker
02176_cvFAP_OH_lox71_rv	CGTATAATGTATGCTATACGAA CGGTACGCGTATATTAGTGAT GATGATGATGATG**TGCTGCAA CTGTCGCC**	amplification of *Cv*FAP from p02010 with overhang to *spec*
02016_specR_lox	TACCGTTCGTATAGCATACATT ATACGAAGTTATT**AGACCGGT ACTAAATTAAAGTAATAAAG**	amplification of *s*pectinomycin resistance cassette with overhang to *lox71*
02017_specR_lox	CTACCGTTCGTATAATGTATG CTATACGAAGTTAT**GGTCAGC TTTATTGAACAGTAATTTAAG**	amplification of *s*pectinomycin resistance cassette with overhang to *lox66*
02021_pksX_back_fw	CATAACTTCGTATAGCATACAT TATACGAACG**GTAGATGAATT GGTGAAGCGCTG**	amplification of backbone with *pksX* integration site from pJK179 with overhang to spec
02020_pksX front_Prom-OH_ rv	GTATTATGTTAAAATGGCCGG AAAATCATGCGGAG**GTAACA AGAAAAAAATGAGAGGG**	amplification of backbone with *pksX* integration site from pJK179 with overhang to P*cotYZ*
02221_cotY_pBReT_fw	AAGCGTTGGTTTGGCAATCTT ATCGGGCTATGCAT**CCAAGC ATATGATGAATATATAGACG**	amplification of *Cv*FAP from Bs02035 genomic DNA, with overhang to high copy plasmid p14037
02222_pksX_pBreT_rv	CGCTCAGTGGAACGAGGCG CCTGATCAACGCGTGC**GGCG TTTGTGTTTTCTCAG**	amplification of *Cv*FAP from Bs02035 genomic DNA, with overhang to backbone for p02027
02219_pBReT_rv	**ATGCATAGCCCGATAAGATT G**	backbone amplification for p02027 from pMSE3 (template)
02220_pBReT_fw	**GCACGCGTTGATCAGGC**	backbone amplification for p02027 from pMSE3 (template)

02177_cvFAP_seq_fw	**ACCGACAGGAGAAAGACTG**	*Cv*FAP sequencing primer
02178_cvFAP_seq_rv	**TTCCAAATTCTTTCAGTTCTGC**	*Cv*FAP sequencing primer

Fig. S1: Custom made photocatalytic set up for small scale production of hydrocarbons. CAD files available at https://gitlab.com/kabischlab.de/led-labware-cvfap

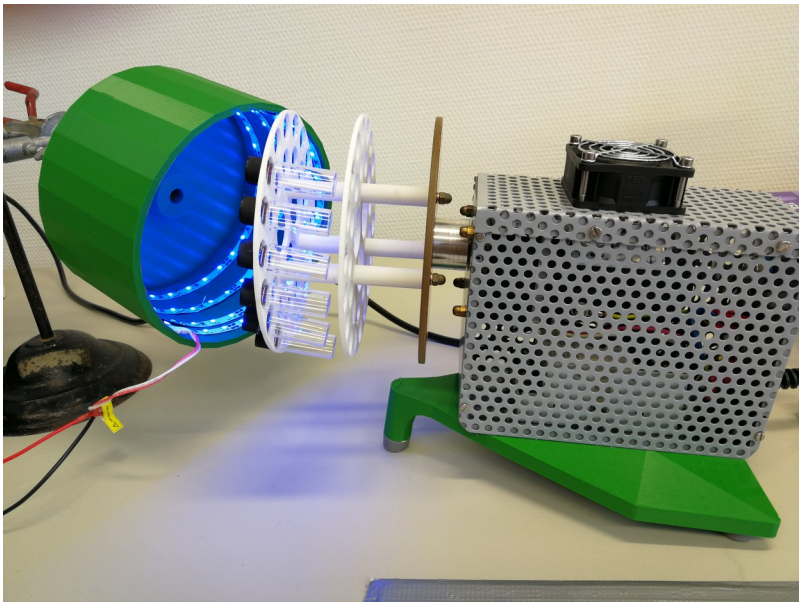


Fig. S2: Effect of light intensity in the enzymatic activity of *Cv*FAP. Reaction conditions: 15 mM palmitic acid, spores Bs02039 (OD_{600} ~ 40), 30 °C, 50 % n-hexane, 70 rpm, blue light illumination.

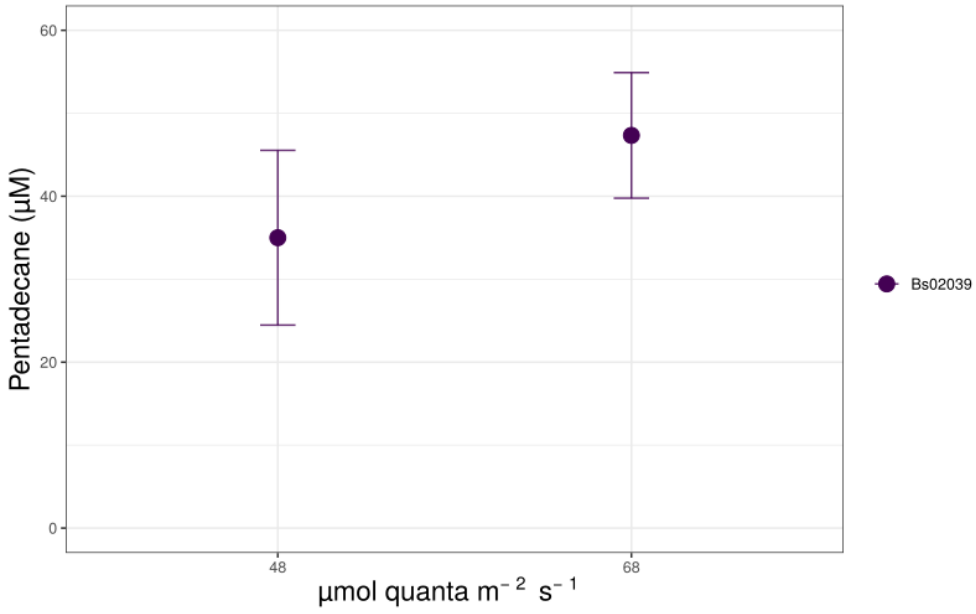


Fig. S3: Time course production of pentadecane from *Cv*FAP using two different substrate concentrations. Reaction conditions: 1.0-2.5 mM palmitic acid, 6 mg/mL of SDW, 30 °C, 25 % n-hexane, 70 rpm, blue light illumination (68 µmol quanta m^{-2} s^{-1})

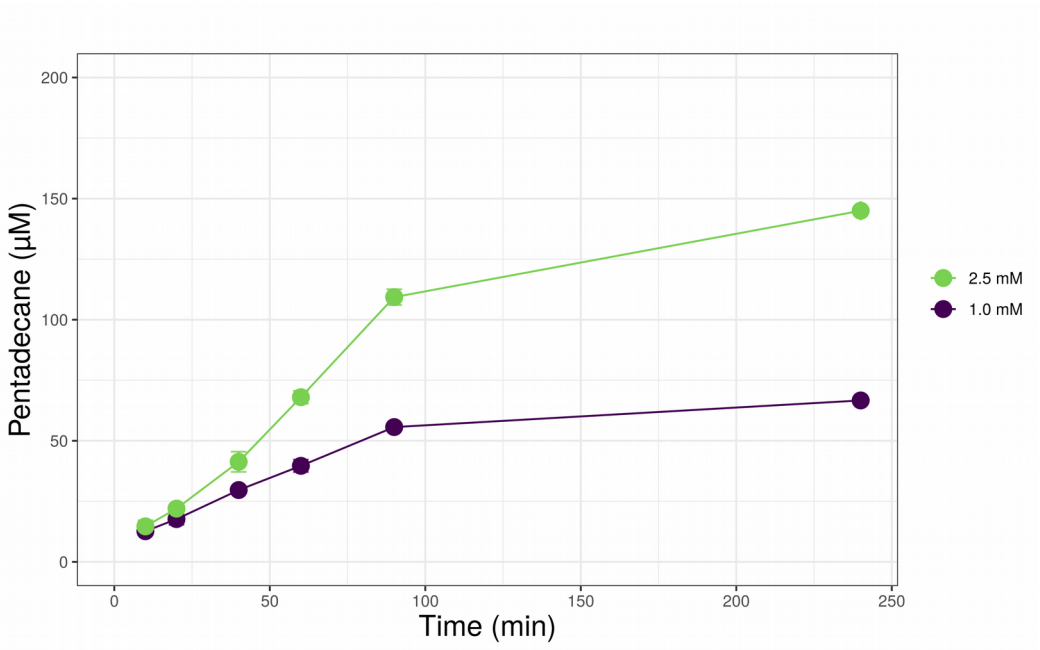


Fig. S4: Kinetic analysis of *Cv*FAP. Michaelis-Menten plot of spore displayed *Cv*FAP (Bs02039) catalysing the decarboxylasion of palmitic acid. Reaction conditions: 0.5-10 mM palmitic acid, 6 mg/mL SDW, 30 °C, 70 rpm, 25 % n-hexane, blue light illumination (68 µmol quanta m^{-2} s^{-1}). Kinetic constants: K_M = 0.96 ± 0.2 mM, V_{max} = 1.36 ± 0.08 µM/min, SDW specific activity = 0.23 µM/min/mg

SDW specific activity = Vmax/[SDW in mg]

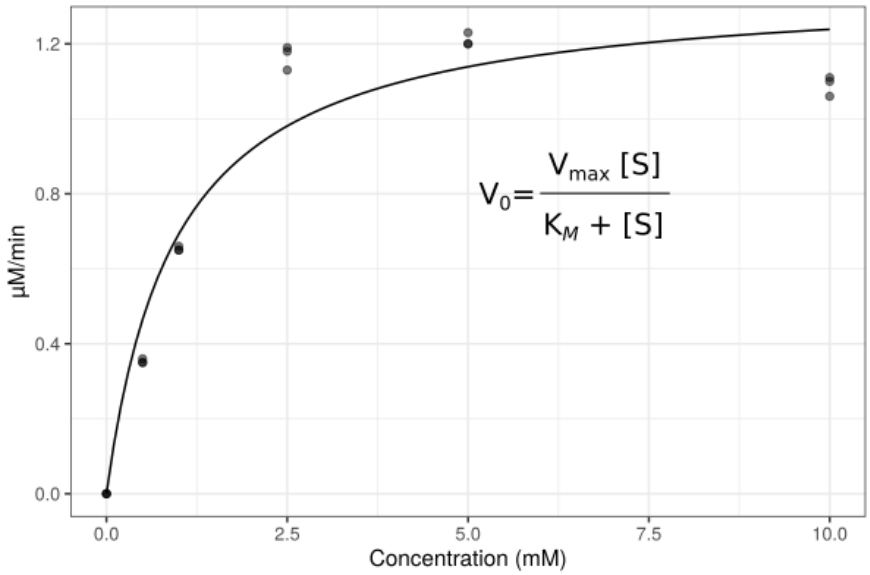


Fig. S5: Relative composition of hydrocarbon titers in percent (%) as produced from the reaction of *Cv*FAP with different oil sources presented in Figure 4.

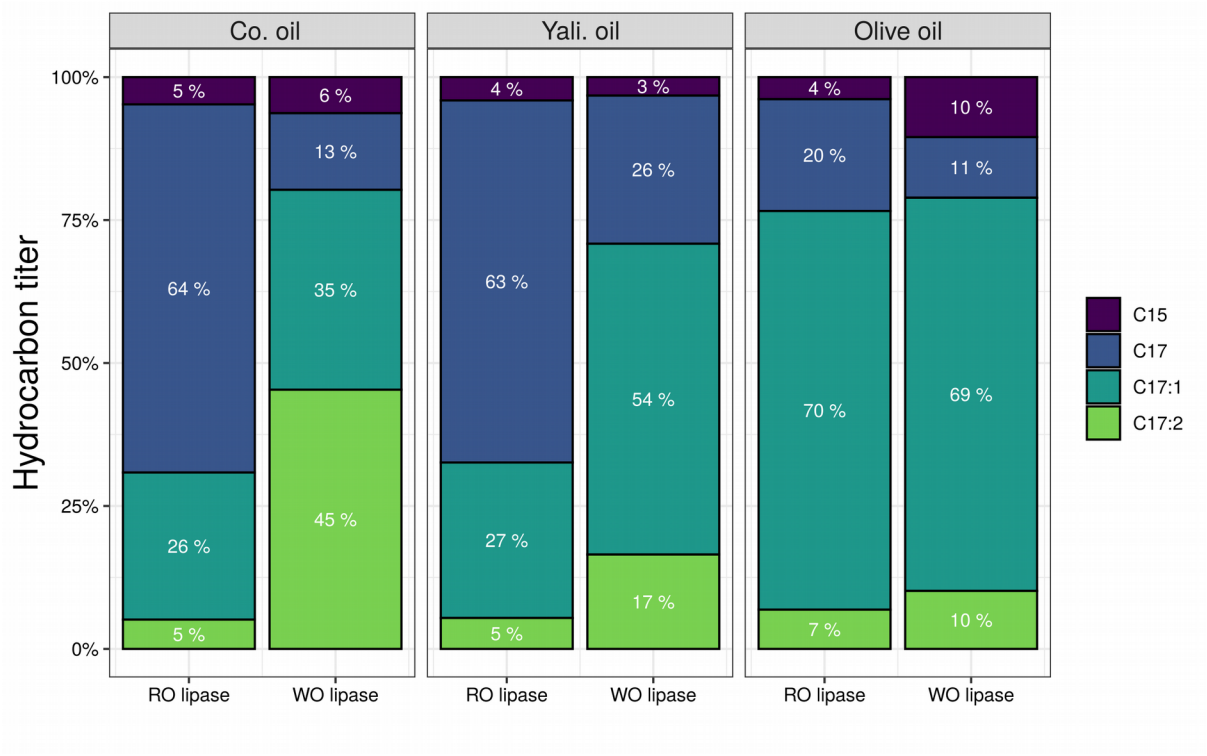


Fig. S6: Produced hydrocarbon titers from the reaction of „wild type“ Bs02003 spores not displaying any heterologous enzyme with different oils. Reaction conditions: 2.5 % v/v oil , Tris-HCl buffer (100 mM, pH 8.5), 16 mg/mL of SDW, 30 °C, 70 rpm, blue light illumination (130 mAmp, 4 V, 68 µmol quanta m^{-2} s^{-1})

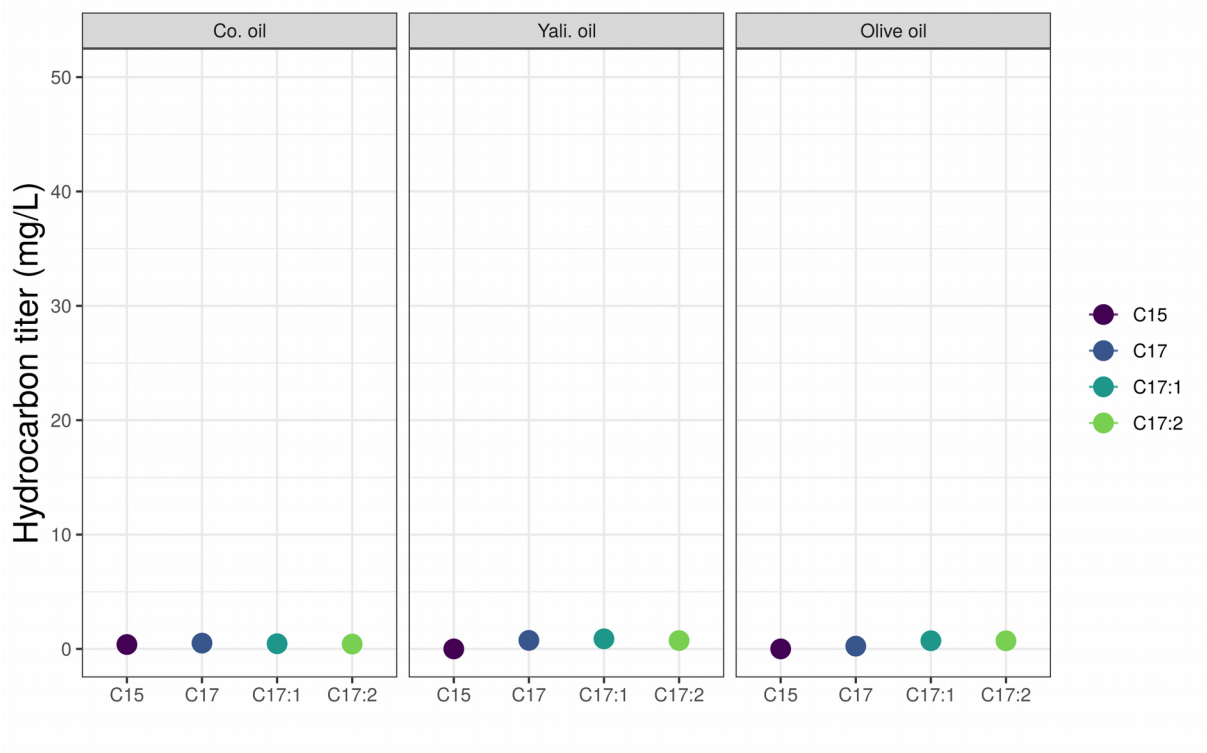


Fig. S7: HPLC chromatograms of employed oils.
Oils were analysed with HPLC (Agilent 1260 infinity bio-inert, Agilent) eqquiped with a luna silica column (250 mm × 4.6 mm i.d., 5 µm particle size, Phenomenex) and a refracive index detector (Shodex-ri 101). Mobilie phase was hexane:isopropanol (18:1), flow was set at 1 mL/min, analysis time was 20 min. Samples were prepared by resuspending 1 µL of oil to 499 µL of mobile phase. The identified peaks correspond to: 1-triglycerides, 2-free fatty acids. Retention times: 3.1 min and 3.7 min respectively. Data were analysed using R 3.6.3.

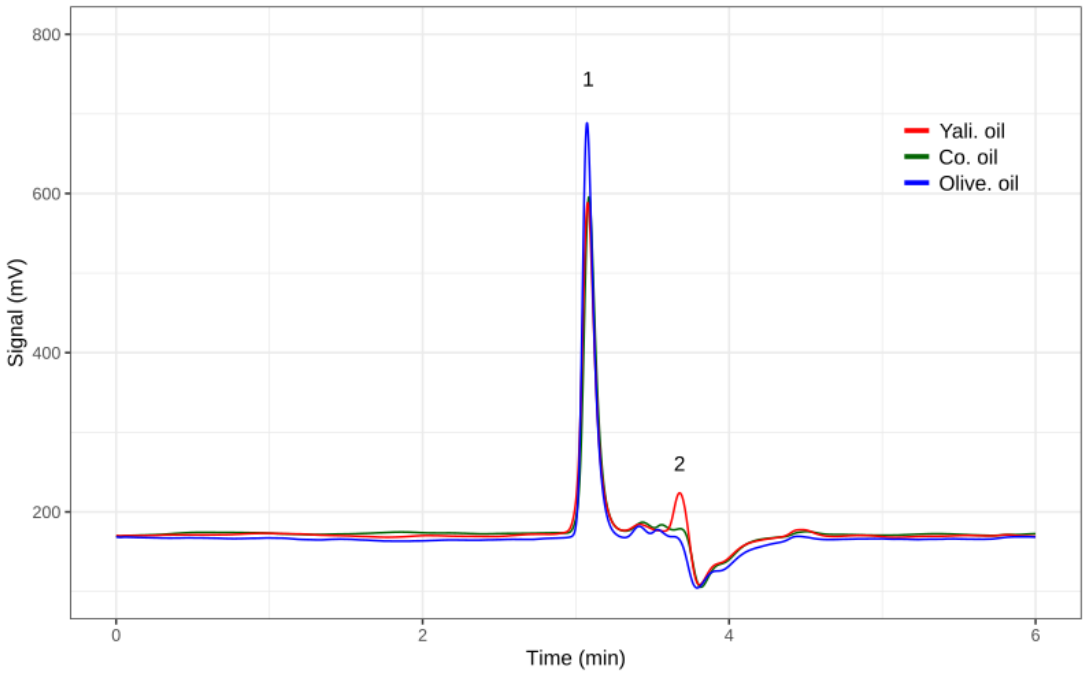
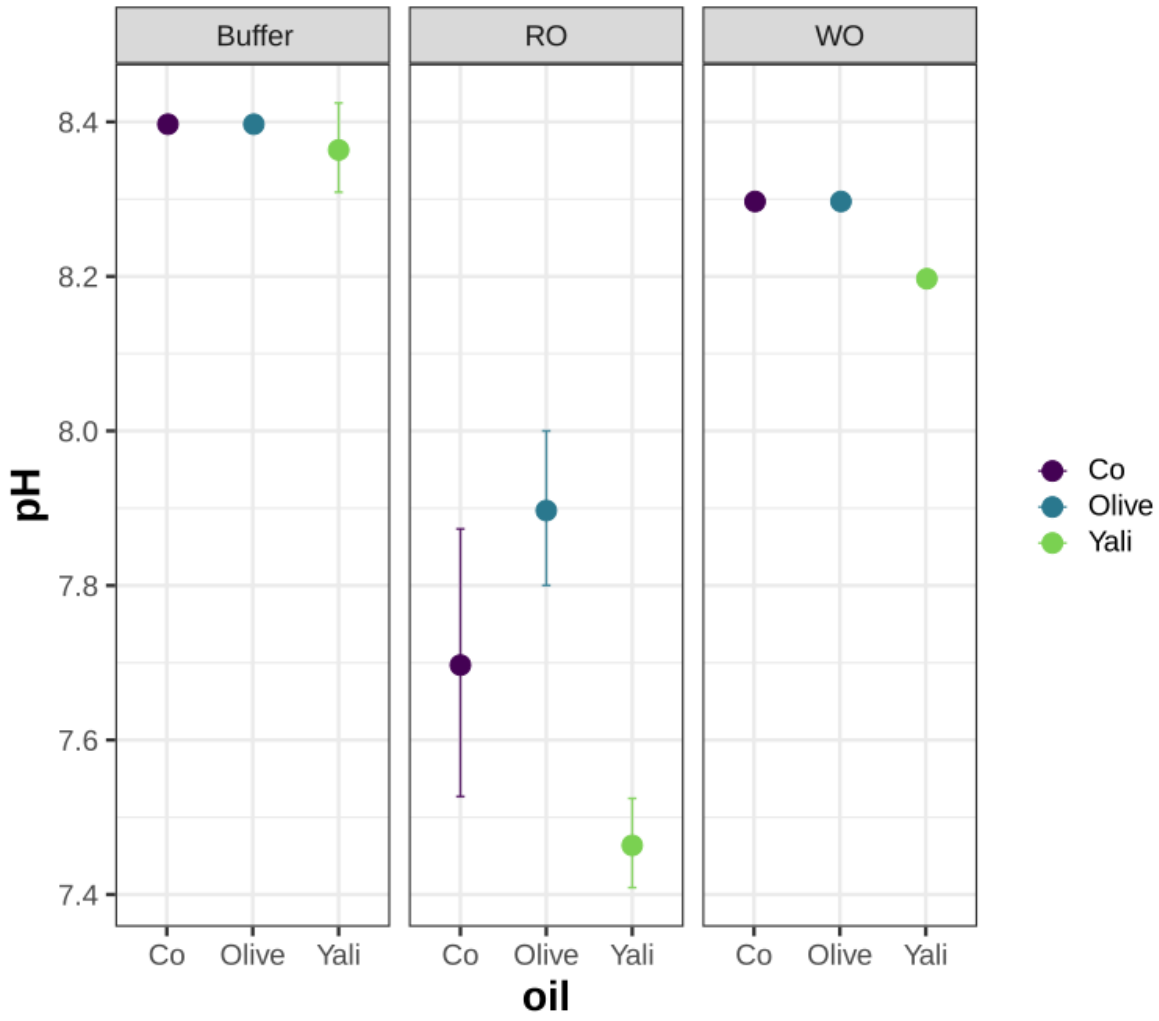


Fig. S8: pH change in reactions containing Yali, Co. and olive oil. Reactions (1 mL) were performed as described in the material and methods section under the one step biotransformation paragraph. All reactions were performed in Tris-HCl buffer (100 mM, pH 8.5) containing oil (2.5 % v/v), spores Bs02039 (16 mg/mL SDW) and RO lipase (50 mg/mL). Buffer: reactions contained oil and buffer, RO: reactions contained spores Bs02039, RO lipase and oil, WO: reactions contained spores Bs02039 and oil.



References

1 M. Karava, F. Bracharz and J. Kabisch, *PLoS One*, 2019, **14**, e0219892.