Electronic Supplementary Material (ESI) for Green Chemistry.

Highly efficient oxidation of plant oils to C18 trihydroxy fatty acids by *Escherichia coli* co-expressing lipoxygenase and epoxide hydrolase†

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Table S1 Purities of plant oxylipins as 11*R*,12*R*,13*S*-TriHOME (**4**) according to purification steps and different types of adsorbent resins

Purification step	Type of adsorbent resin	Purity (%)
Cell reaction[a]		75.1
Prep LC		87.4
Resin	SP825	94.0
	SP850	89.3
	HP20	88.1
	SP207	90.1
	HP2MG	88.4

[a] 11*R*,12*R*,13*S*-TriHOME as plant oxylipin were obtained from the conversion of LA by *E. coli* co-expressing *A. violaceum* LA 13*S*-LOX and *M. xanthus* EH with or without cysteine.

Table S2 Regression equations for the calibration curves of the peak areas in HPLC chromatograms to the amount of PUFAs, HFAs, HpFAs, EHFAs, and THFAs

Type	Compound	Regression equation[a]	r^2
PUFAs	**1**	$y = 0.0000389x - 0.0031$	0.9919
	6	$y = 0.0000601x - 0.0312$	0.9883
	11	$y = 0.0000382x - 0.0035$	0.9894
HpFAs and HFAs[b]	**2** and **5**	$y = 0.0001328x - 0.0027$	0.9908
	7 and **10**	$y = 0.0001314x + 0.0015$	0.9971
	12 and **15**	$y = 0.0001351x - 0.0006$	0.9987
EHFAs[c]	**3**	$y = 0.0001090x + 0.0075$	0.9903
	8	$y = 0.0001462x + 0.0071$	0.9950
	13	$y = 0.0001341x + 0.0068$	0.9934
THFAs[d]	**4**	$y = 0.000903x - 0.015$	0.9921
	9	$y = 0.000928x + 0.020$	0.9812
	14	$y = 0.000102x - 0.001$	0.9864

HFAs, hydroxy fatty acids; EFHAs, epoxy hydroxy fatty acids; THFAs, trihydroxy fatty acids; LA (**1**), linoleic acid; ALA (**6**), α-linolenic acid; GLA (**11**), γ-linolenic acid; 13*S*-HODE (**5**), 13*S*-hydroxy-9*Z*,11*E*-octadecadienoic acid; 13*S*-HOTrE (**10**), 13*S*-hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid; 13*S*-HOTrE(γ) (**15**), 13*S*-hydroxy-6*Z*,9*Z*,11*E*-octadecatrienoic acid; 12*S*,13*S*-EHOME (**3**), 12*S*,13*S*-epoxy-11*R*-hydroxy-9*Z*-octadecenoic acid; 12*S*,13*S*-EHODE (**8**), 12*S*,13*S*-epoxy-11*R*-hydroxy-9*Z*,15*Z*-octadecadienoic acid; 12*S*,13*S*-EHODE(γ) (**13**), 12*S*,13*S*-epoxy-11*R*-hydroxy-6*Z*,9*Z*-octadecadienoic acid; 11*R*,12*R*,13*S*-TriHOME (**4**), 11*R*,12*R*,13*S*-trihydroxy-9*Z*-octadecenoic acid; 11*R*,12*R*,13*S*-TriHODE (**9**), 11*R*,12*R*,13*S*-trihydroxy-9*Z*,15*Z*-octadecadienoic acid; 11*R*,12*R*,13*S*-TriHODE(γ) (**14**), 11*R*,12*R*,13*S*-trihydroxy-6*Z*,9*Z*-octadecadienoic acid. [a] *x*, peak area in the HPLC profile; *y*, molar concentration of standard (mM). [b] HFAs or HpFAs were obtained from the conversion of C18 PUFAs by *E. coli* expressing LA 13*S*-LOX from *A. violaceum* with or without cysteine as a reducing agent, respectively. The concentrations of HFAs calibrated by the peak areas were the same as those of HpFAs.[1] [c] EHFAs were obtained from the conversion of C18 PUFAs by *E. coli* expressing *A. violaceum* LA 13*S*-LOX without cysteine. [d] THFAs were obtained from the conversion of C18 PUFAs by *E. coli* co-expressing *A. violaceum* LA 13*S*-LOX and *M. xanthus* EH without cysteine.

Table S3 Specific activities and kinetic parameters of LOXs for isomerization of PUFAs into HpFAs and EHFAs

Microorganism	Enzyme	Substrate	Specific activity [μmol per mg cells per min][a]	K_m [μM]	k_{cat} [s^{-1}]	k_{cat}/K_m [s^{-1} μM^{-1}]	Reference
Archagium violaceum	13*S*-LOX	**1**	31.6 ± 0.4	30.7	76.6	2.49	This study
		6	22.9 ± 0.2	198	149	0.75	
		11	11.9 ± 0.1	161	96.2	0.59	
		ARA	28.4 ± 0.2	68.5	164.3	2.39	[2]
		EPA	15.4 ± 0.3	58.2	32.6	0.56	
		DHA	18.8 ± 0.2	33.7	31.8	0.94	
		DPA	14.0 ± 0.4	65.8	22.9	0.34	
Burkholeria thailandensis	13*S*-LOX	**1**	26.4	41.5	93.7	2.26	[3]
		6	10.5	59.8	42.0	0.70	
		11	2.9	88.1	13.6	0.15	
Myxococcus xanthus	9*S*-LOX	**1**	11.4 ± 0.2	330	7.95	0.024	[4]
		6	3.4 ± 0.1	81.0	1.27	0.016	
		11	1.9 ± 0.3	73.0	0.81	0.011	

LA (**1**), linoleic acid; ALA (**6**), α-linolenic acid; GLA (**11**), γ-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid. [a] The specific activities were determined using a spectrometer by measuring the absorbance at 234 nm of the reaction solutions obtained from the conversion of C18 PUFAs. Data are presented as mean ± SD (n=3) values.

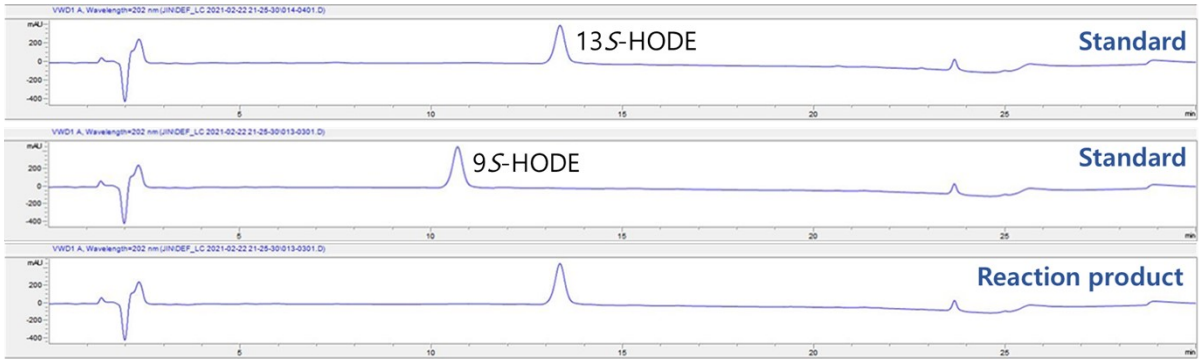
Table S4 Specific activities of *E. coli* cells expressing *A. violaceum* LA 13*S*-LOX and its purified LA 13*S*-LOX for C18 PUFAs

Substrate	Product	Recombinant cells	Purified 13*S*-LOX
		Specific activity [nmol per mg cells per min]	Specific activity [nmol per mg enzyme per min]
1	**2** and **5**	75.2 ± 0.7	1303 ± 3.3
6	**7** and **10**	72.2 ± 2.1	1000 ± 3.8
11	**12** and **15**	29.4 ± 1.3	359 ± 1.2

LA (**1**), linoleic acid; ALA (**6**), α-linolenic acid; GLA (**11**), γ-linolenic acid; 13*S*-HpODE (**2**), 13*S*-hydroperoxy-9*Z*,11*E*-octadecenoic acid; 13*S*-HODE (**5**), 13*S*-hydroxy-9*Z*,11*E*-octadecadienoic acid; 13*S*-HpOTrE (**7**), 13*S*-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid; 13*S*-HOTrE (**10**), 13*S*-hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid; 13*S*-HpOTrE(γ) (**12**), 13*S*-hydroperoxy-6*Z*,9*Z*,11*E*-octadecatrienoic acid; 13*S*-HOTrE(γ) (**15**), 13*S*-hydroxy-6*Z*,9*Z*,11*E*-octadecatrienoic acid. The specific activities were determined by HPLC at an absorbance wavelength of 202 nm. Data are presented as mean ± SD (n=3) values.

Supporting Figures

A



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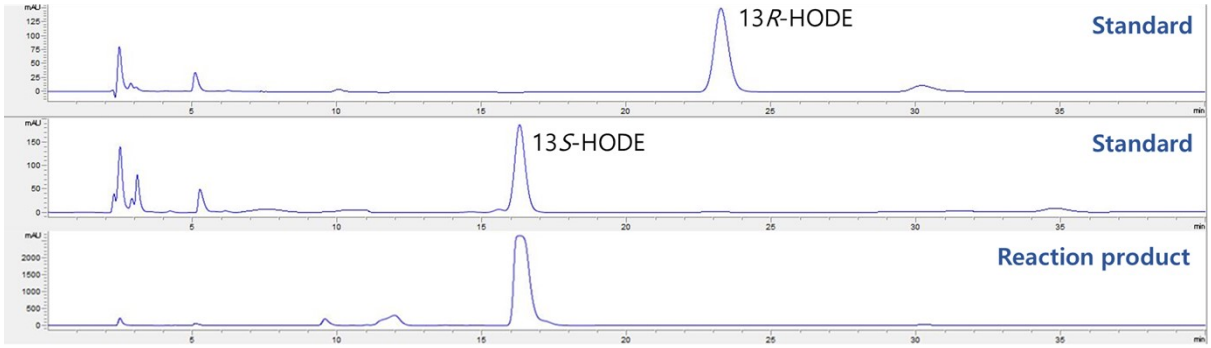
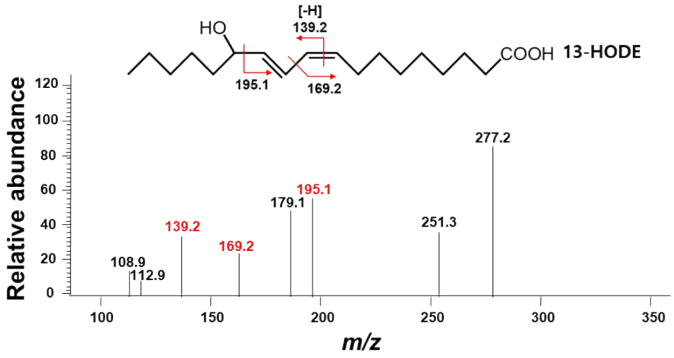


Fig. S1 HPLC chromatograms of the product obtained from the conversion of LA (**1**) by purified *A. violaceum* LA 13*S*-LOX in the presence of cysteine as a reducing agent with 13*S*-, 13*R*-, and 9*S*-HODE as standards. The reaction product was identified as 13*S*-HODE (**5**). (A) Reverse-phase HPLC profiles of the product compared to 13*S*-HODE (**5**) and 9*S*-HODE standards. (B) Chiral phase-HPLC profiles of the product compared to 13*S*- and 13*R*-HODE standards. The reactions were performed at 30 °C in 50 mM HEPPS buffer (pH 8.5) containing 1 mM **1** 0.5 g L^{-1} enzyme, and 10 mM cysteine for 10 min with shaking at 200 rpm. The HpFA 13*S*-HpODE was reduced to 13*S*-HODE by cysteine during the biotransformation. LA (**1**), linoleic acid; HODE (**5**), hydroxyoctadecenoic acid.

A



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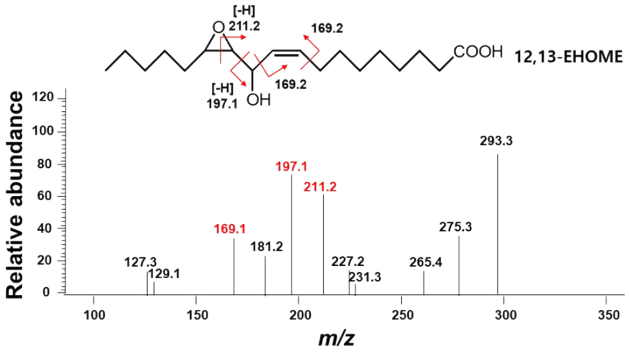

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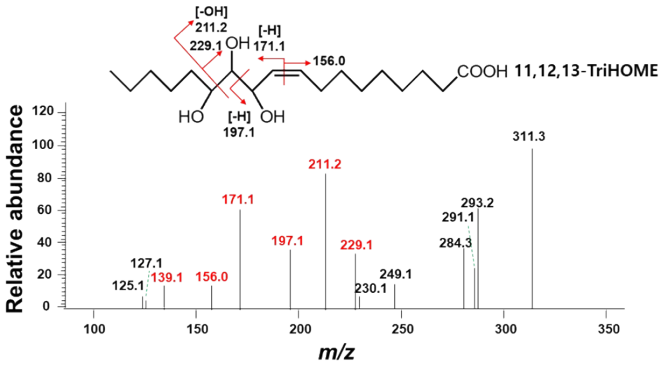
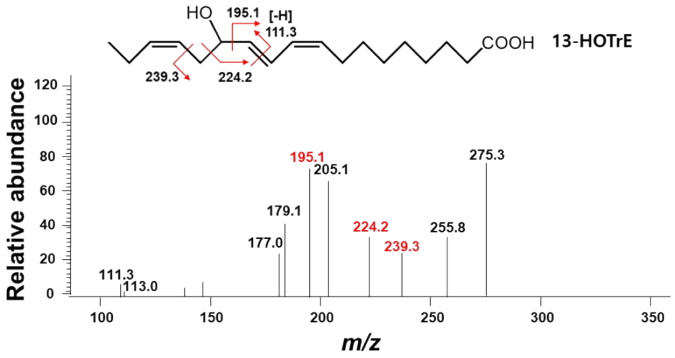


Fig. S2 LC-MS/MS analysis of the products obtained from the conversion of LA (**1**) by *E. coli* co-expressing *A. violaceum* LA 13*S*-LOX and *M. xanthus* EH. (A) **5** (ESI-MS *m/z*: 295.6 [M−H$^-$]). (B) **3** (ESI-MS *m/z*: 311.2 [M−H$^-$]). (C) **4** (ESI-MS *m/z*: 329.5 [M−H$^-$]). The reactions were performed without cysteine. The red arrows and numbers indicate critical fragments around the functional groups and mass per charge (*m/z*) values of the main peaks, respectively.

A



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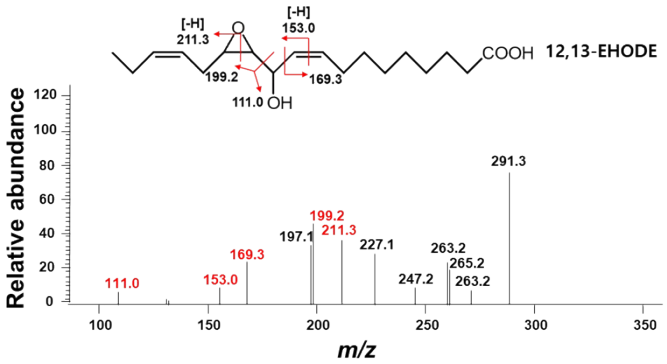

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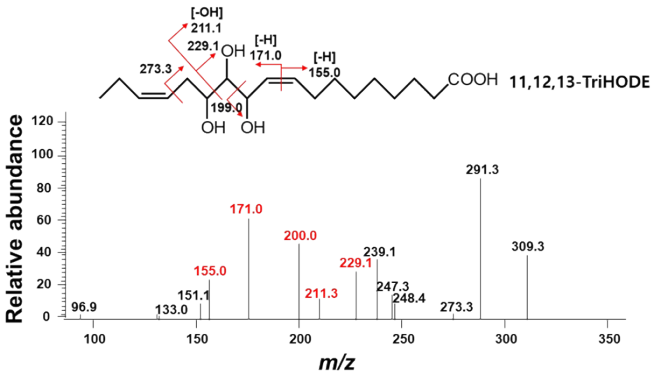
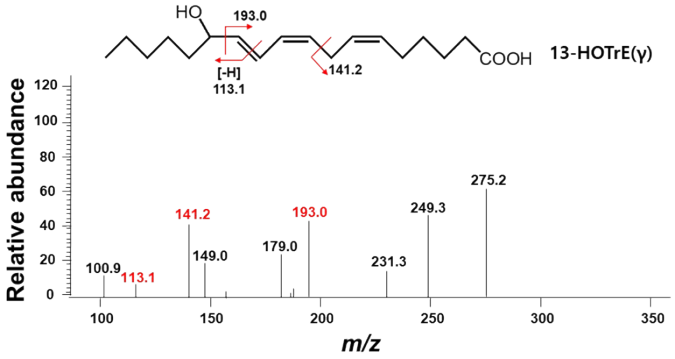


Fig. S3 LC-MS/MS analysis of the products obtained from the conversion of ALA (**6**) by *E. coli* co-expressing *A. violaceum* LA 13*S*-LOX and *M. xanthus* EH. (A) **10** (ESI-MS *m/z*: 293.6 [M−H$^-$]). (B) **8** (ESI-MS *m/z*: 309.6 [M−H$^-$]). (C) **9** (ESI-MS *m/z*: 327.6 [M−H$^-$]). The reactions were performed without cysteine. The red arrows and numbers indicate critical fragments around the functional groups and mass per charge (*m*/*z*) values of the main peaks, respectively.

A



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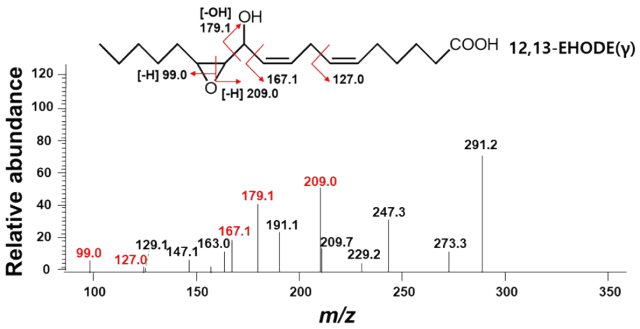

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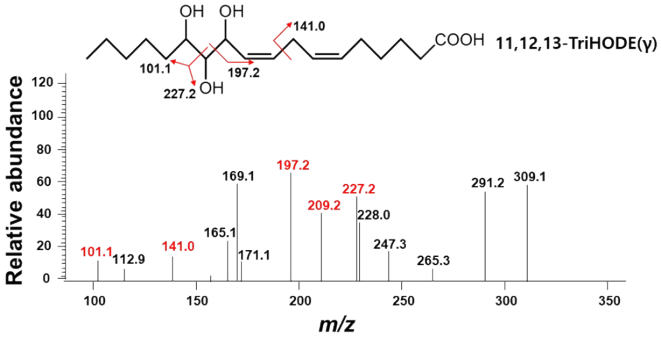
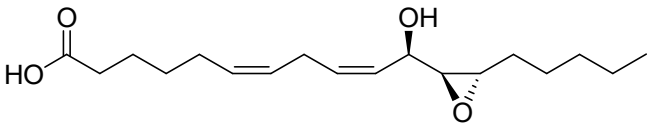


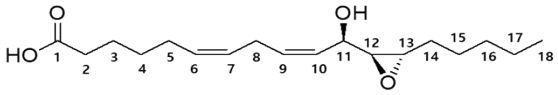
Fig. S4 LC-MS/MS analysis of the products obtained from the conversion of GLA (**11**) by *E. coli* co-expressing *A. violaceum* LA 13*S*-LOX and *M. xanthus* EH. (A) **15** (ESI-MS *m/z*: 293.6 [M−H$^-$]). (B) **13** (ESI-MS *m/z*: 309.7 [M−H$^-$]). (C) **14** (ESI-MS *m/z*: 327.8 [M−H$^-$]). The reactions were performed with cysteine. The red arrows and numbers indicate critical fragments around the functional groups and mass per charge (*m*/*z*) values of the main peaks, respectively.



(*R*,6*Z*,9*Z*)-11-hydroxy-11-((2*S*,3*S*)-3-pentyloxiran-2-yl)undeca-6,9-dienoic acid

Fig. S5 Verification of the structure of 12*S*,13*S*-EHODE(γ) (**13**) by NMR. 12*S*,13*S*-EHODE(γ), 12*S*,13*S*-epoxy-11*R*-hydroxy-6*Z*,9*Z*-octadecadienoic acid.

A



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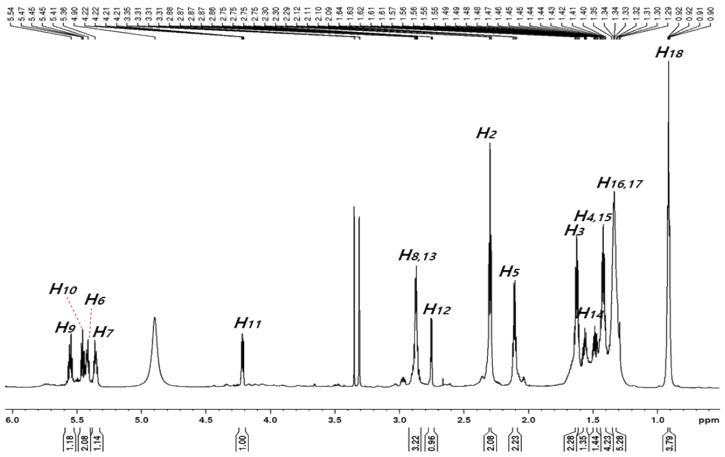

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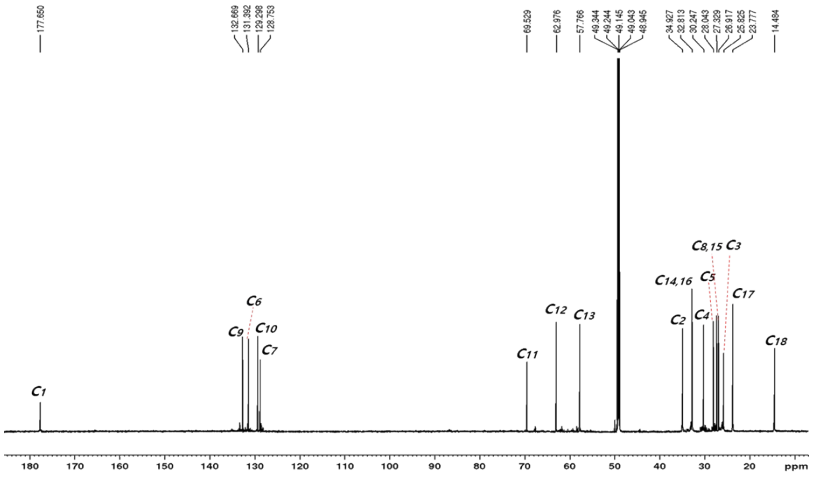


Fig. S6 1D NMR profiles of 12*S*,13*S*-EHODE(γ) (**13**) (850 MHz NMR). (A) 1H NMR peak of **13** in $CDCl_3$. (B) 13C NMR peak of **13** in $CDCl_3$.

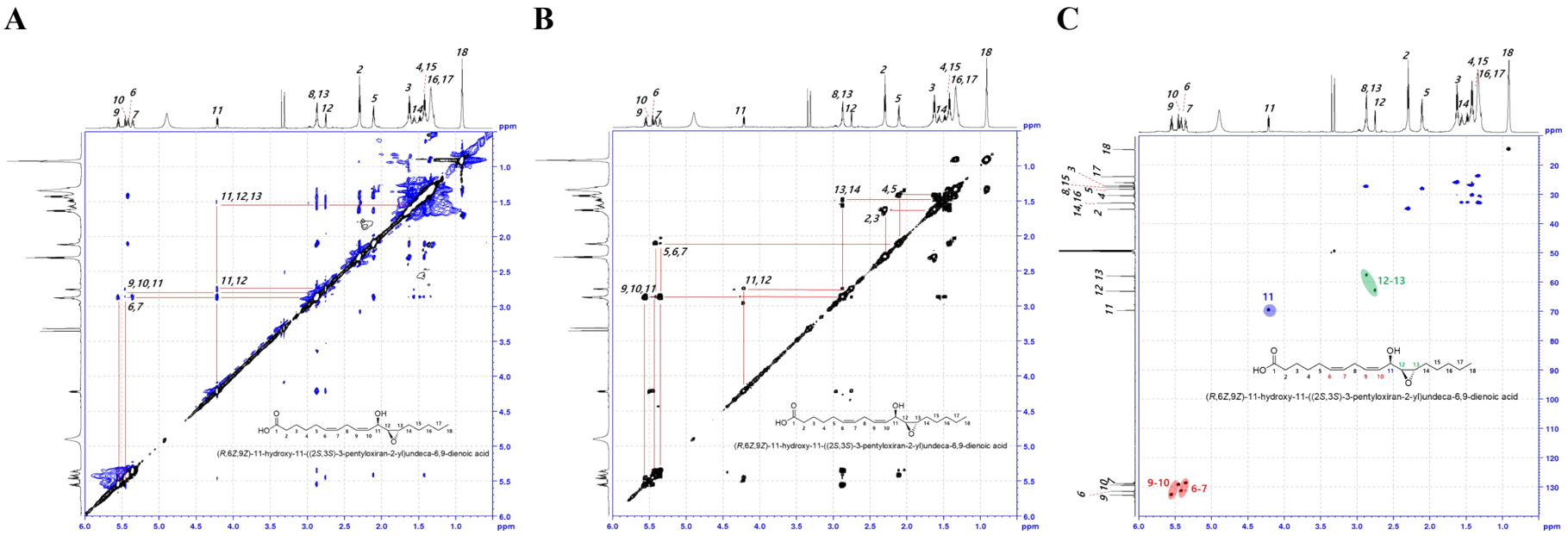
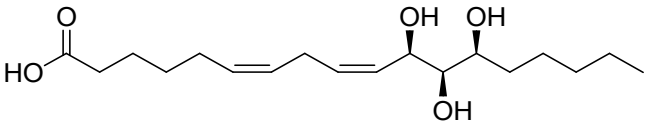


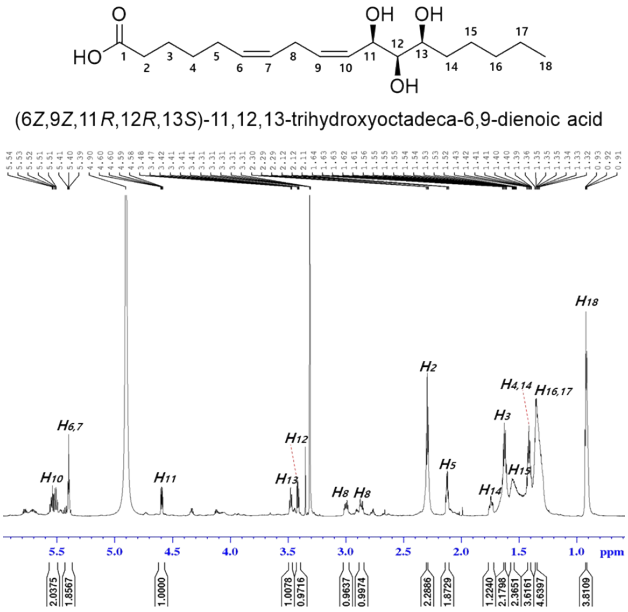
Fig. S7 2D NMR profiles of 12*S*,13*S*-EHODE(γ) (**13**) (850 MHz NMR). (A) ROSEY profile. (B) COSY profile. (C) HSQC profile.



(6*Z*,9*Z*,11*R*,12*R*,13*S*)-11,12,13-trihydroxyoctadeca-6,9-dienoic acid

Fig. S8 Verification of the structure of 11*R*,12*R*,13*S*-TriHODE(γ) (**14**) by NMR. 11*R*,12*R*,13*S*-TriHODE(γ), 11*R*,12*R*,13*S*-trihydroxy-6*Z*,9*Z*-octadecadienoic acid.

A



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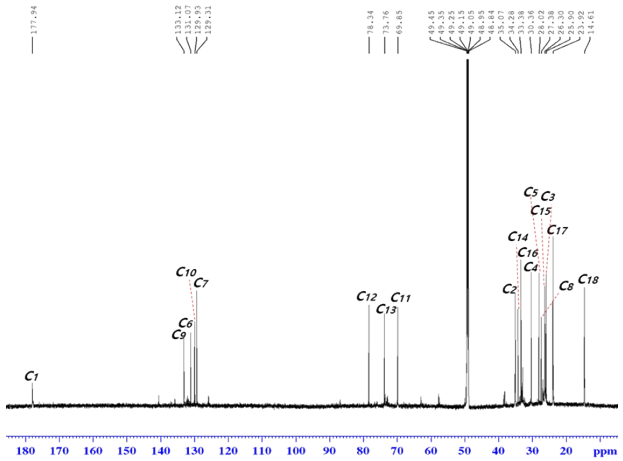

Fig. S9 1D NMR profiles of 11*R*,12*R*,13*S*-TriHODE(γ) (**14**) (850 MHz NMR). (A) 1H NMR peak of **14** in $CDCl_3$. (B) 13C NMR peak of **14** in $CDCl_3$.

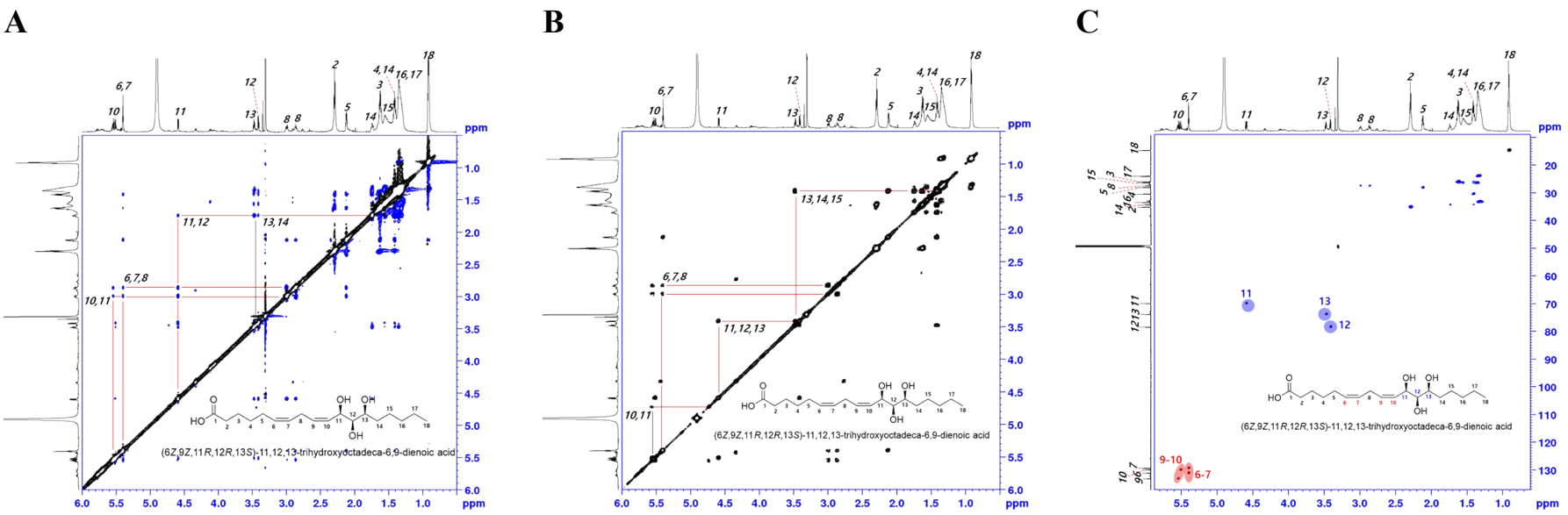

Fig. S10 2D NMR profiles of 11*R*,12*R*,13*S*-TriHODE(γ) (**14**) (850 MHz NMR). (A) ROSEY profile. (B) COSY profile. (C) HSQC profile.

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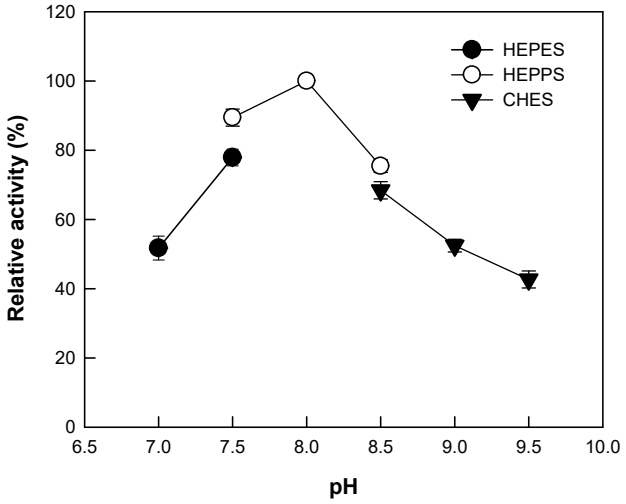
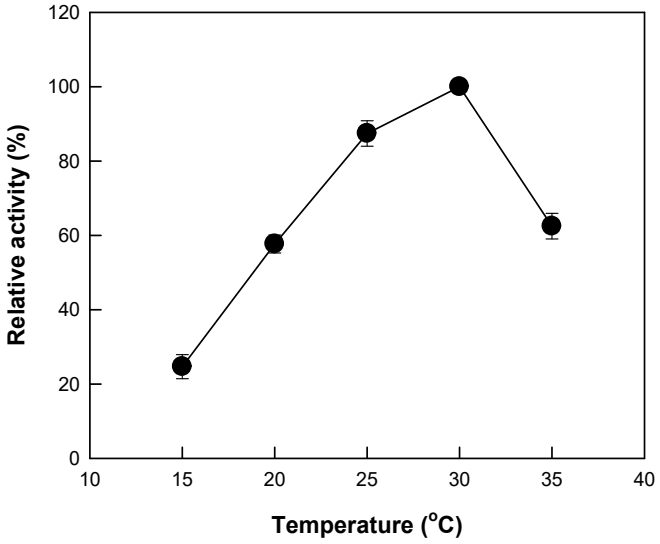
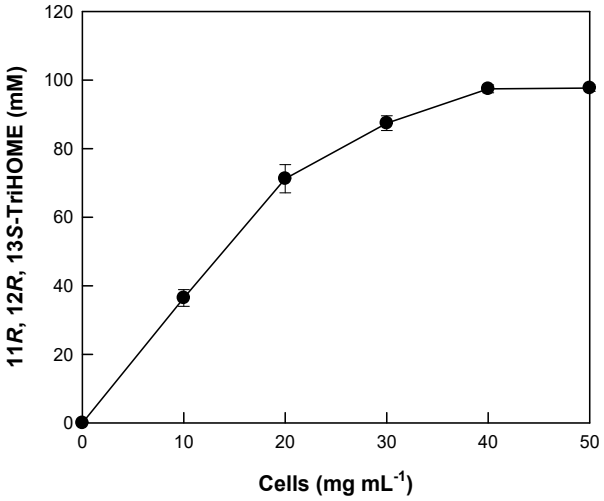

HEPES
HEPPS
CHES
Relative activity (%)
pH

B


Relative activity (%)
Temperature (°C)

C


11R, 12R, 13S-TriHOME (mM)
Cells (mg mL-1)

D

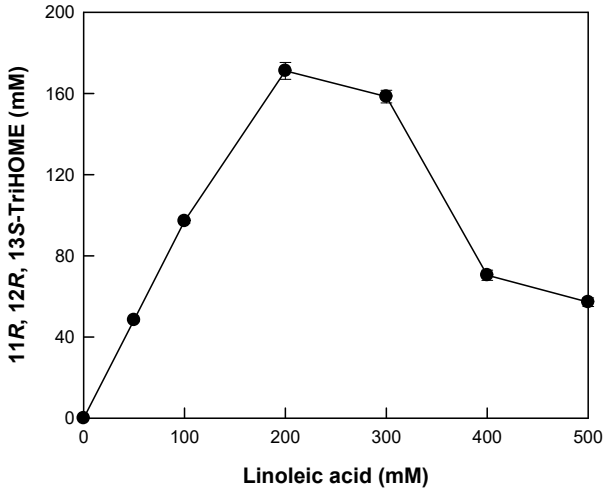


Fig. S11 Effects of pH, temperature, and cell and substrate concentration on the production of 11*R*,12*R*,13*S*-TriHOME (**4**) by *E. coli* co-expressing *A. violaceum* LA 13*S*-LOX and *M. xanthus* EH. (A) Effect of pH on the production of **4**. The reactions were performed at 30 °C in 50 mM HEPES (pH 7.0-7.5; ●), 50 mM HEPPS (pH 7.5-8.5; ○), or 50 mM CHES (pH 8.5-9.5; ▼**)** buffer containing 1 mM **1**, 0.5 mg mL^{-1} cells, and 5 % (v/v) methanol for 30 min in a 500-mL baffled flask containing 100 mL reaction solution. (B) Effect of temperature on the production of **4**. The reactions were performed in 50 mM HEPPS buffer (pH 8.0) containing 1 mM **1**, 0.5 mg mL^{-1} cells, and 5 % (v/v) methanol with shaking at 200 rpm for 30 min in a 500-mL baffled flask containing 100 mL reaction solution. (C) Effect of cell concentration. The reactions were performed at 30 °C in 50 mM HEPPS buffer (pH 8.0) containing 100 mM **1** and 5 % (v/v) methanol with shaking at 200 rpm for 24 h in a 500-mL baffled flask containing 100 mL reaction solution. (D) Effect of substrate concentration on the production of **4**. The reactions were performed at 30 °C in 50 mM HEPPS (pH 8.0) buffer containing 40 mg mL^{-1} cells and 5 % (v/v) methanol with shaking at 200 rpm for 24 h in a 500-mL baffled flask containing 100 mL reaction solution. Data are presented as mean ± SD (n=3) values.

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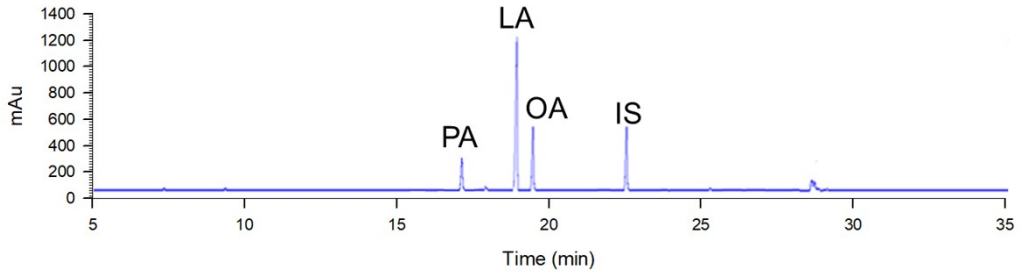

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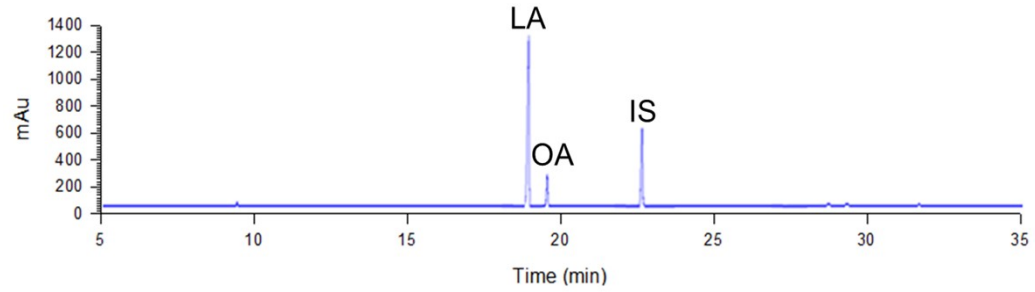

Fig. S12. GC profiles of resin-untreated safflower oil hydrolyzate and resin-treated safflower oil hydrolyzate. (A) GC profile of resin-untreated safflower oil hydrolyzate. (B) GC profile of resin-treated safflower oil hydrolyzate. The adsorbent resin bound LA and OA but not glycerol or PA, and the bound LA and OA were recovered from the resin by ethyl acetate extraction.

References

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2 J. Lee, J. U. An, T. H. Kim, Y. J. Ko, J. B. Park and D. K. Oh, *ACS Sustain. Chem. Eng.*, 2020, **8**, 16172-16183.

3 J. U. An, B. J. Kim, S. H. Hong and D. K. Oh, *Appl. Microbiol. Biotechnol.*, 2015, **99**, 5487-5497.

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