

Structural characterization of mono- and dimethylphosphatidylethanolamines from various organisms using a complex analytical strategy including chiral chromatography

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1. Supplementary Data

Materials and Methods

Chemicals and standards

Phospholipase C from *Bacillus cereus* (≥ 200 units/mg protein), preparative TLC Glass Plates L $\times$ W 20 cm $\times$ 20 cm, silica gel 60 matrix with polymeric binder and fluorescent indicator, O-16:0/18:1-PC, 1-O-hexadecyl-2-oleoyl-sn-glycero-3-phosphocholine (878112P-5mg), O-16:0/18:1-PE, 1-O-hexadecyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (878130C-5mg), O-16:0/O-16:0-PC, 1,2-di-O-hexadecyl-sn-glycero-3-phosphocholine (999992P-10mg), P-18:0/18:1-PC, 1-O-1'-(Z)-octadecenyl-2-oleoyl-sn-glycero-3-phosphocholine (852467C-5mg), P-18:0/18:1-PE, 1-O-1'-(Z)-octadecenyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (852758P-5mg), P-18:0/18:1-PC, 1-O-1'-(Z)-octadecenyl-2-oleoyl-sn-glycero-3-phosphocholine (852467C-5mg), 16:0/18:1-PC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (850457C-25mg), 16:0/18:1-PE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (850757P-25mg), 3-palmitoyl-2-oleoyl-sn-glycero-1-phosphocholine (850855C-5mg), 16:0/16:0-DMPE, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N,N-dimethyl (850854P-25mg), 16:0/16:0-MMPE, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-methyl (850851P-25mg), 1-hydroxy-2-oleoyl-sn-glycero-3-phosphocholine (855773C-1mg), 16:0/18:1-PI, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoinositol (ammonium salt) (850142P-500 μ g), P-16-LPC, 1-O-1'-(Z)-hexadecenyl-sn-glycero-3-phosphocholine (852464P-10mg), commercial bacterial FAMES standard solution (BAME), PUFA No.1 Marine source, PUFA No.2 (Animal Source), PUFA No.3 from Menhaden Oil, analytical standards and all other reagents used were of analytical grade were purchased from Merck (previously Sigma-Aldrich and/or Supelco, Prague, Czech Republic).

Cultivation

The anaerobic beer spoiling bacteria *Megasphaera cerevisiae* (DSM 20461) and *Pectinatus frisingensis* (DSM 20465) used in this study were obtained from the German collection

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of microorganisms and cell cultures (DSMZ, Braunschweig, Germany). All microorganisms were cultivated in 52.2 g/L MRS broth medium (Merck, Darmstadt, Germany) with a typical composition (g/L): peptone from casein 10.0, meat extract 8.0, yeast extract 4.0, D(+)-glucose 20.0, di-potassium hydrogen phosphate 2.0, Tween 80 1.0, di-ammonium hydrogen citrate 2.0, sodium acetate 5.0, magnesium sulfate 0.2, manganese sulfate 0.04. The MRS broth was further supplemented with (g/L) (R)-(+)-Cysteine hydrochloride hydrate 0.25 and sodium thioglycolate 0.25. The first cultivation was carried out in a microbiology test tube (5 mL), while the subsequent cultures were in unshaken flasks with increasing volumes 50, 250 and 800 mL. Each subsequent culture was inoculated with the sedimented biomass of the previous one. All cultivations were carried out in an anaerobic incubator (30 °C, 72 hours, N₂ atmosphere).

The unicellular red alga *Galdieria sulphuraria* (Galdieri) Merola ACUF 002 (Algal Collection of Dipartimento delle Scienze Biologiche, Section of Plant Biology, University “Federico II” of Naples, Italy) and the green alga *Desmodesmus quadricauda* (Turpin) Brébisson CCALA 463 (previously *Scenedesmus quadricauda*, strain Greifswald/15) from the Culture Collection of Autotrophic Organisms, Institute of Botany of the Czech Academy of Sciences, Třeboň, Czech Republic, were grown in glass cylinder photobioreactors (200 mL). The photobioreactors were placed in a thermostatic unit and illuminated by a panel of dimmable fluorescent lamps (OSRAM DULUX L55 W/950 Daylight, Milano, Italy). The algal cultures in the photobioreactors were supplied with a mixture of air and CO₂ gas (2% v/v), at a flow rate of 15 L h⁻¹. The experiments were carried out in a batch culture regime, at temperatures of 30 °C and 40 °C for *D. quadricauda* and *G. sulphuraria*, respectively and incident light intensity of 500 µmol photons m⁻² s⁻¹. *G. sulphuraria* was grown in modified Galdieria-nutrient medium pH 3 [1] and *D. quadricauda* was grown in SS-medium pH 7 [2]. The biomass was harvested by centrifugation (ROTINA 380R, HETTINGER, Tuttleingen, Germany) at 5000 rpm for 10 min, frozen at -80 °C and then freeze dried (CoolSafe 95-15Pro, Labogene, Lillerød, Denmark).

The mold *Aspergillus niger* 629 CCF was obtained from the Culture Collection of Fungi, Faculty of Science, Charles University in Prague, and maintained on yeast medium slants (0.3% malt extract, 0.3% yeast extract, 0.5% peptone, and 0.5% glucose) at 4 °C. Production of conidia by the fungal strain was performed on yeast medium plates at 30 °C for 7 days until fully conidiated. For analysis of lipids, the fungal strain was grown in minimal medium (in g/L: NaCl 0.1, K₂HPO₄ 1.03, KH₂PO₄ 0.75, and NH₄Cl 1.0) containing 0.5% glucose as the carbon source and trace elements (in mg/L: MgSO₄ × 7H₂O 200, CaCl₂ × 2H₂O 10, FeSO₄ × 7H₂O 1.5). The culture was inoculated with conidia (10⁶/mL) and incubated at 28 °C and 150 rpm for three days.

Fatty acid methyl esters (FAME) and dimethylacetals (DMA) analysis

Fatty acids and fatty aldehydes were released from total lipids according to the previously published paper [3]. Briefly, the total lipids were treated with acid (2 M HCl, 100 °C, 2.5 h), extracted with hexane and methylated with 10 % (w/v) methanolic BF₃ (80 °C, 10 min). GC-MS of FAME and DMA mixtures were carried out on a Finnigan 1020 B in EI mode. Splitless injection was at 100 °C, and a fused silica capillary column (Supelcowax 10; 60 m × 0.25 mm i.d., 0.25 mm film thickness; Supelco, Prague) was used. The temperature program was as follows: 100 °C for 1 min, subsequently increasing at 20 °C/min to 180 °C and at 2 °C/min to 280 °C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm/s. All spectra were scanned within the range of m/z 50–500. The structures of FAMEs were confirmed by comparison of retention times and fragmentation patterns with those of the standard FAMEs (Sigma-Aldrich, Prague) [4,5].

Separation FAME and DMA by TLC and reduction of DMA

The FAME and DMA mixtures were separated on a silica gel plate (20 × 20 cm) and the elution was performed using CH₂Cl₂ [3]. Bands (FAME R_f = 0.60, DMA R_f = 0.38) were visualized (UV light) by spraying with 2,7-dichlorofluorescein in methanol, scraped and extracted from silica by hexane. After evaporation of hexane in a stream of nitrogen, the DMA were acid hydrolyzed (2 M HCl, 25 °C, 2.5 h) and extracted with hexane.

Reduction of aldehydes

Alcohols were prepared according to Farquhar [6]. Briefly, the free aldehydes were dissolved in a 1 mL of anhydrous ethyl ether, cooled to -20 °C, and 3 mL of a 3% solution of LiAlH₄ in anhydrous ether was added with constant stirring. The reaction proceeded for 2 hours at -20 °C, a mixture of 90% methanol-water was added to decompose the excess LiAlH₄, the precipitate was centrifuged, washed with ether, and evaporated to dryness under vacuum.

Preparation of nicotines of long chain fatty alcohols

N,N'-dicyclohexylcarbodiimide (5 mg) was added to a solution of a mixture of fatty alcohols (0.5 mg), nicotinic acid (2 mg) and 4-dimethylaminopyridine (0.5 mg) in dichloromethane (3 mL) at 0 °C for 5 minutes. The mixture was cooled to room temperature and then left overnight. Hexane (2 mL) was then added and the product was extracted and further analyzed by GC-MS [7].

Preparation of 3-pyridylcarbonyl esters of FAs

Transesterification of FAME into 3-pyridylcarbinol esters was performed according to Destailats and Angers [8]. Briefly, a solution of potassium tert-butoxide in THF (100 µL, 1.0 M) was added to 3-hydroxymethylpyridine (200 µL). FAME (1 mg) was added with stirring in dry dichloromethane (1 mL) and the mixture was held at 35 °C for 15 minutes. An aqueous solution of sodium bicarbonate (1 mL, 2.5%) was added and the organic phase was directly used for GC-MS.

GC-MS of 3-pyridylcarbonyl ester of fatty acids and nicotines of long chain fatty alcohols

A fatty acid 3-pyridylcarbonyl ester mixture was analyzed on the instrument described above. Injection temperature (splitless injection) was 100 °C, and an Ultra-1 capillary column (Supelco, Prague, Czech Republic) was used. The temperature program was as follows: 100 °C for 1 min, subsequently increasing at 20 °C/min to 180 °C and at 2 °C/min to 280 °C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm/s. All spectra were scanned within the range of m/z 50–500.

2. Supplementary Figures and Tables

2.1. Supplementary Figures

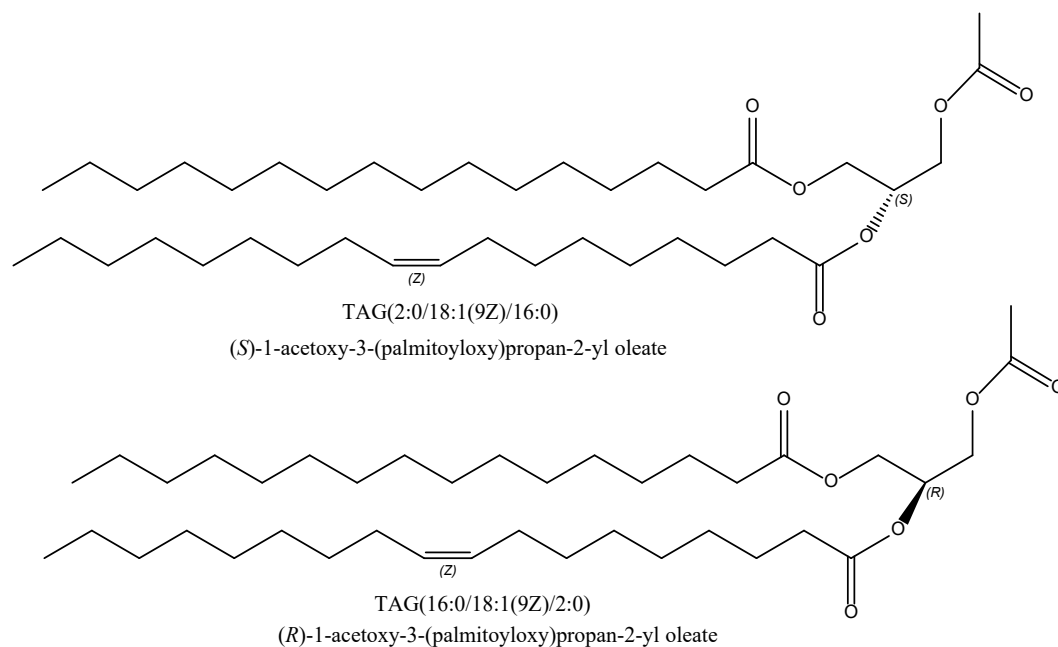


Figure S1: Structure of two enantiomeric TAG

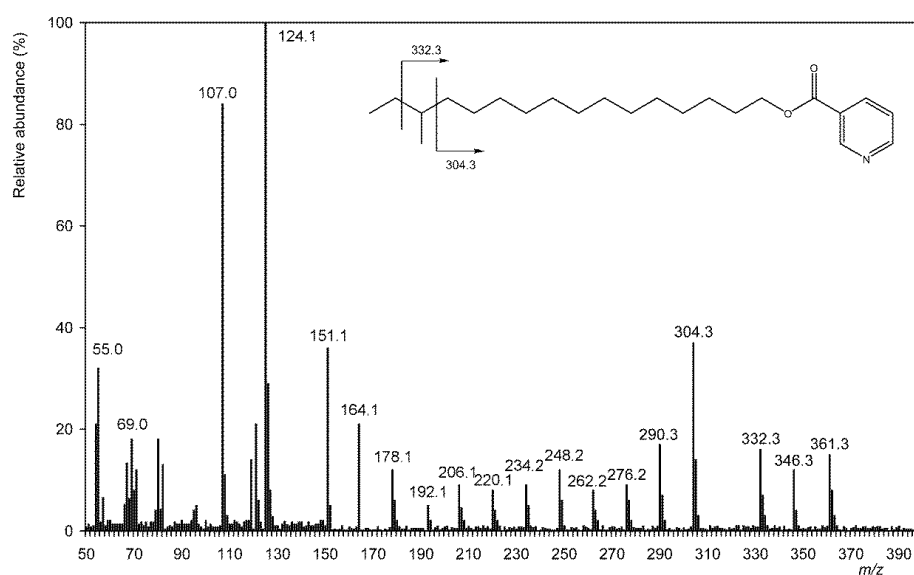


Figure S2: Mass spectrum of nicotinate ester of 14-methylhexadecanol

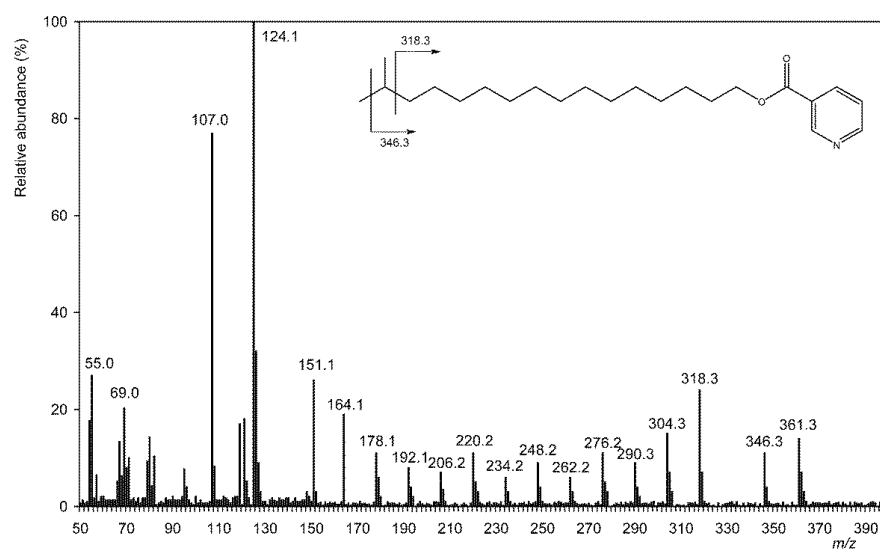


Figure S3: Mass spectrum of nicotinate ester of 15-methylhexadecanol

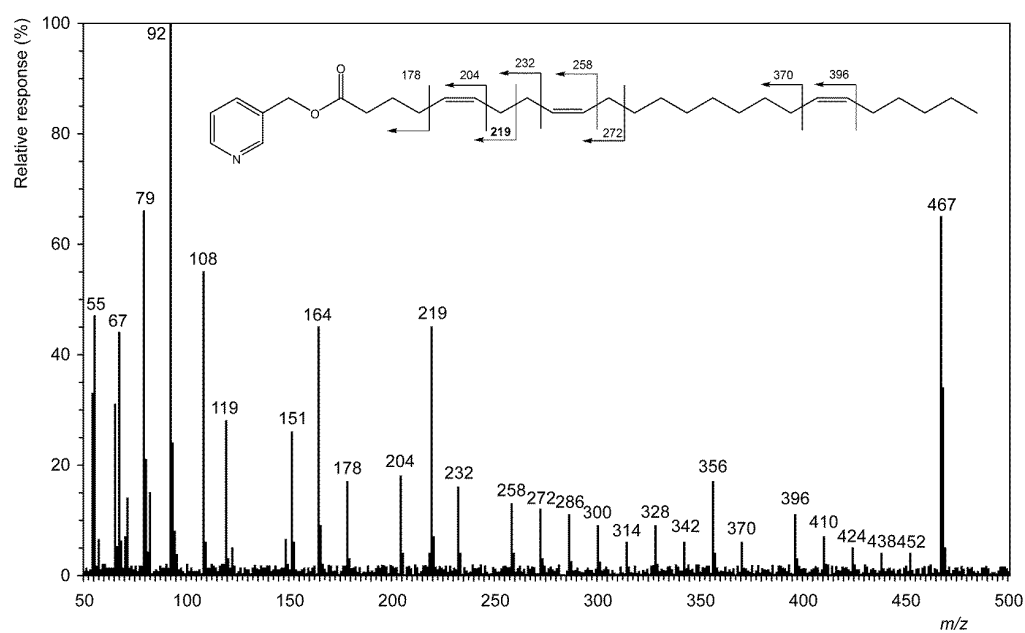


Figure S4: Mass spectrum of 3-pyridylcarbonyl 5,9,19-pentacosatrienoate

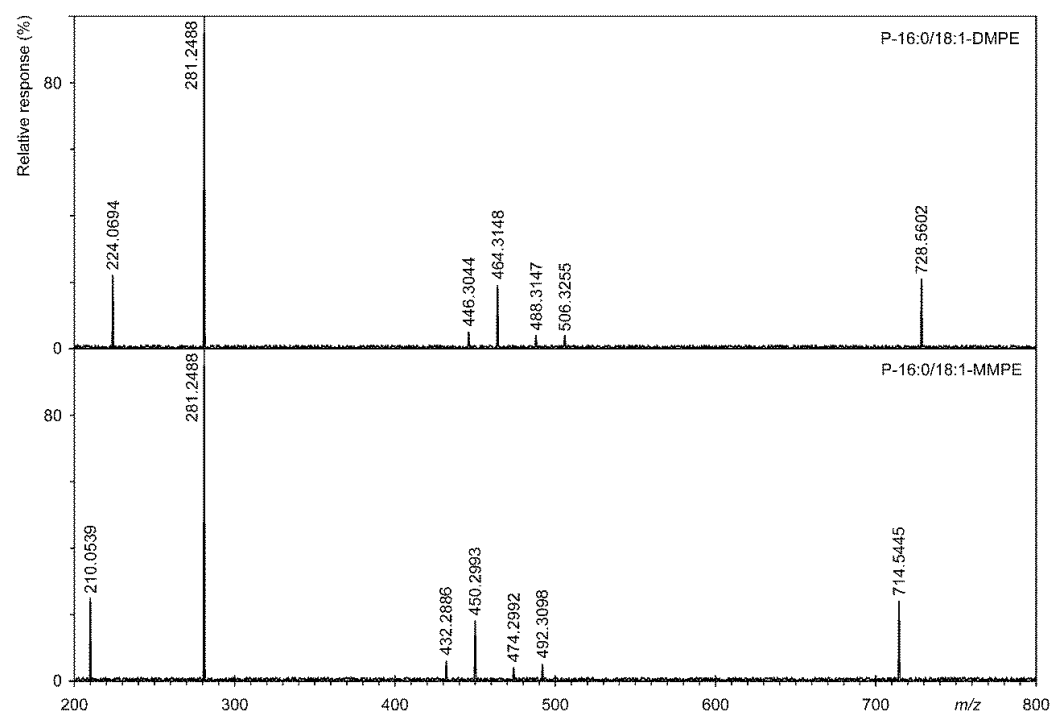


Figure S5: Tandem MS of ions at m/z 728.5601 and 714.5443 ($[M-H]^-$). Based on the m/z values for the ions, see Tables S2 and S3, P-16:0/18:1-DMPE and 714.5443 P-16:0/18:1-MMPE, the probable structures have been proposed

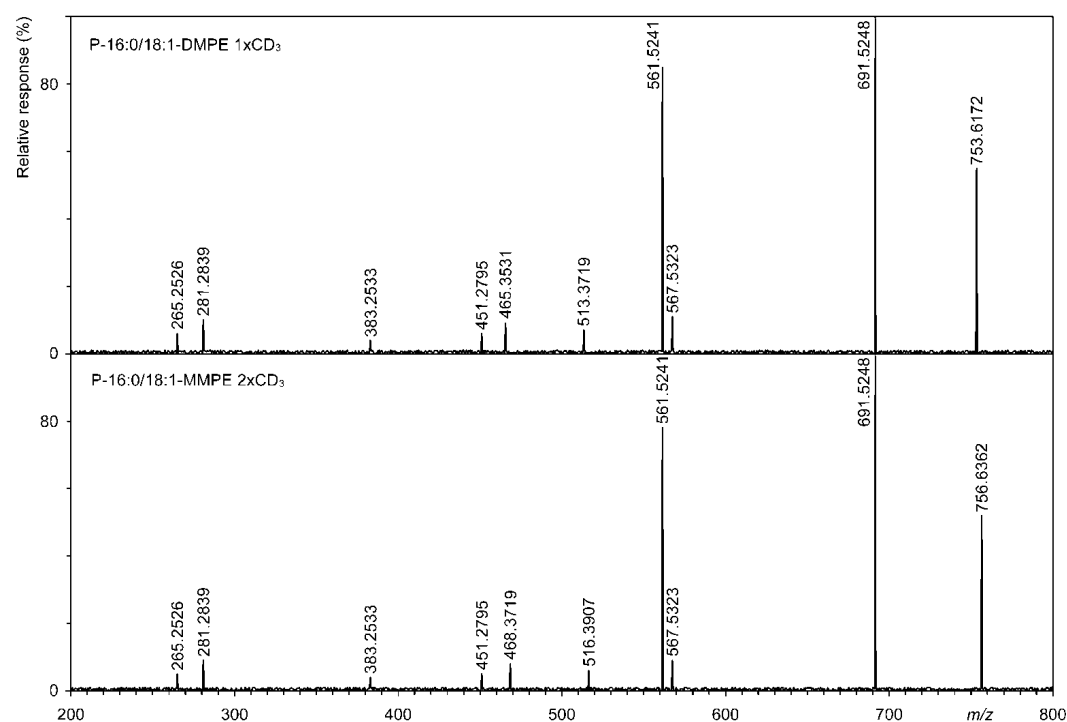


Figure S6: Tandem mass spectra (positive ESI) of lithium adducts of deuterium-labeled natural phospholipids; 1-alkenyl-2-acyl-PC (P-16:0/18:1-DMPE with $1 \times CD_3$ and P-16:0/18:1-MMPE with $2 \times CD_3$)

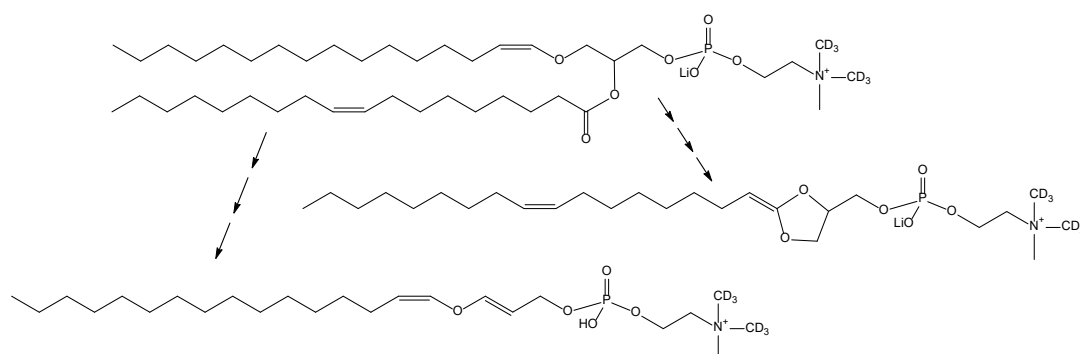


Figure S7: Structures of deuterated methyl-PE key ions (P-16:0/18:1-methyl-PE with 2 × CD₃) obtained by tandem MS

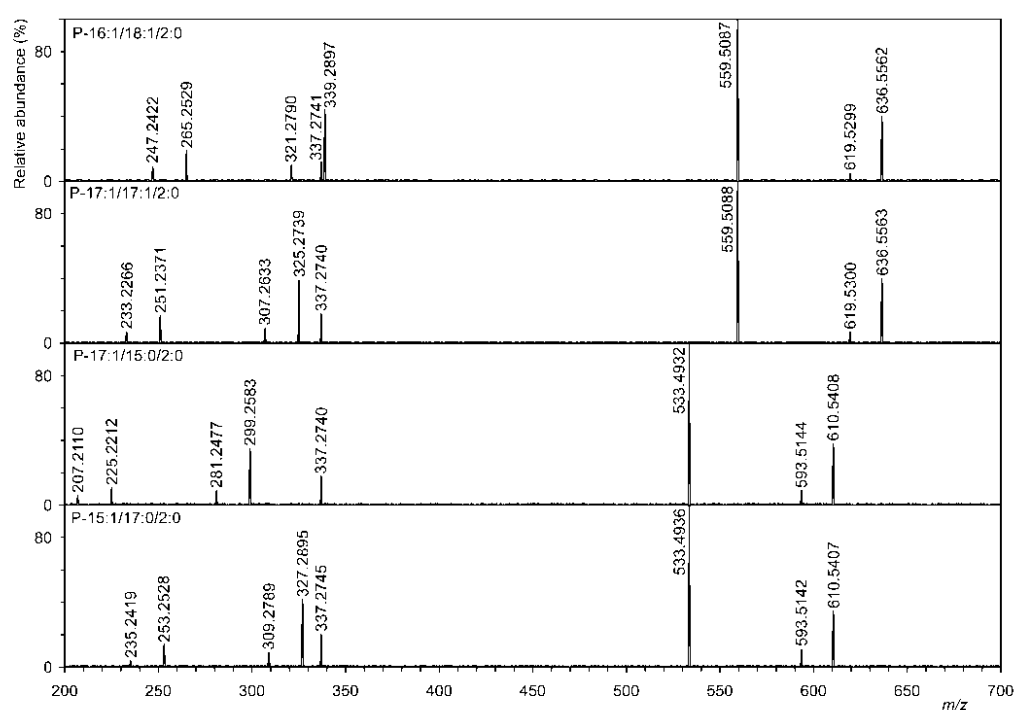


Figure S8: Tandem mass spectra of four AcTAG (P-16:1/18:1/2:0, P-17:1/17:1/2:0, P-17:1/15:0/2:0, P-15:1/17:0/2:0,) obtained from two beer spoilage bacteria *Pectinatus* and *Megasphaera*

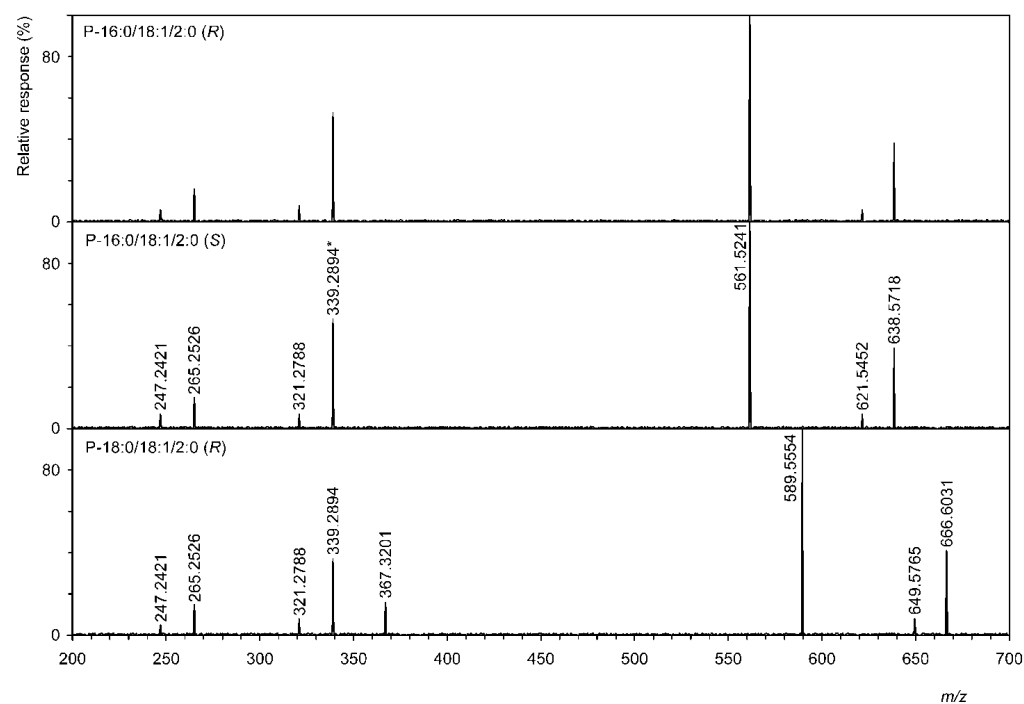


Figure S9: Tandem mass spectra of two enantiomers, i.e. two molecular species P-16:0/18:1/2:0 and 2:0/18:1/P-16:0, and the synthetic derivative (P-18:0/18:1/2:0)

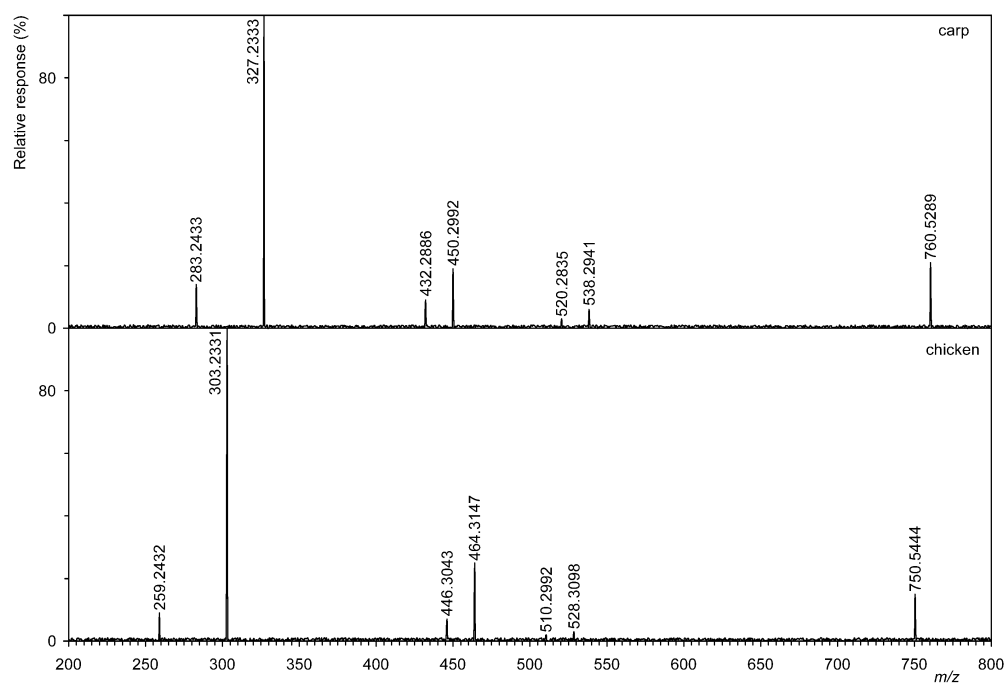


Figure S10: Tandem mass spectra of two molecular species, i.e. molecular species P-16:0/22:6-methyl-PE (carp) and P-16:0/20:4-dimethyl-PE (chicken).

2.2. Supplementary Tables

Table S1: Examples of organisms and their proteins involvement in lipid biosynthesis

Organisms	Protein name	GenBank ID
<i>Arthrobacter</i> sp. ^A	glycerol-1-phosphate dehydrogenase	SDP79711.1
	glycerol-3-phosphate dehydrogenase	KUM38942.1
<i>Aspergillus niger</i>	phosphatidyl- <i>N</i> -methylethanolamine <i>N</i> -methyltransferase	TPR01769.1
Bovine heart	phosphatidylethanolamine <i>N</i> -methyltransferase	XP_027372981.1
Carp	phosphoethanolamine <i>N</i> -methyltransferase (predicted)	XP_018963479.1
<i>Galdieria sulphuraria</i>	phosphatidylethanolamine <i>N</i> -methyltransferase	EME26291.1
Chicken	phosphatidylethanolamine <i>N</i> -methyltransferase	XP_015149665.1
<i>Kocuria palustris</i>	phosphoethanolamine <i>N</i> -methyltransferase	KUG54981.1
<i>Kocuria rhizophila</i>	phospholipid methyltransferase	TDP57698.1
	glycerol-3-phosphate dehydrogenase	KUP28229.1
<i>Lactobacillus iners</i>	glycerol-1-phosphate dehydrogenase	PMC46187.1
<i>Laetiporus fungus</i> ^B	phosphatidyl- <i>N</i> -methylethanolamine <i>N</i> -methyltransferase	PCH43169.1
<i>Lucifera butyrica</i> ^C	<i>sn</i> -glycerol-1-phosphate dehydrogenase	WP_122628282.1
<i>Megasphaera cerevisiae</i>	phospholipid <i>N</i> -methyltransferase	SKA05810.1
	glycerol-3-phosphate dehydrogenase	WP_048513017.1
<i>Pectinatus</i>	SAM-dependent methyltransferase	WP_132547387
	anaerobic glycerol-3-phosphate dehydrogenase	WP_132549284.1
	<i>sn</i> -glycerol-1-phosphate dehydrogenase	WP_132548237.1
<i>Raoultella ornithinolytica</i>	phospholipid <i>N</i> -methyltransferase	STR72523.1
	glycerol-3-phosphate dehydrogenase	STR68378.1
<i>Saccharomyces pastorianus</i>	phosphatidylethanolamine <i>N</i> -methyltransferase	QID85214.1
<i>Salmonella enterica</i> ^D	<i>sn</i> -glycerol-1-phosphate dehydrogenase	AVB03899.1
<i>Scenedesmus</i> ^E	phosphatidyl- <i>N</i> -methylethanolamine <i>N</i> -methyltransferase	JABVCE010000016.1
Spinach	phosphoethanolamine <i>N</i> -methyltransferase-like	XP_021840090.1
<i>Spongilla lacustris</i> ^F	phosphoethanolamine <i>N</i> -methyltransferase-like	XP_019851130.1

Color background - protein catalyzing dehydrogenation of substrate

Color background - protein catalyzing transfer of methyl group

^A *Arthrobacter* and also *Kocuria* belong to the same family, i.e. Micrococcaceae

^B Family Polyporaceae is the same for both fungi, i.e. *Wolfiporia* and *Laetiporus*

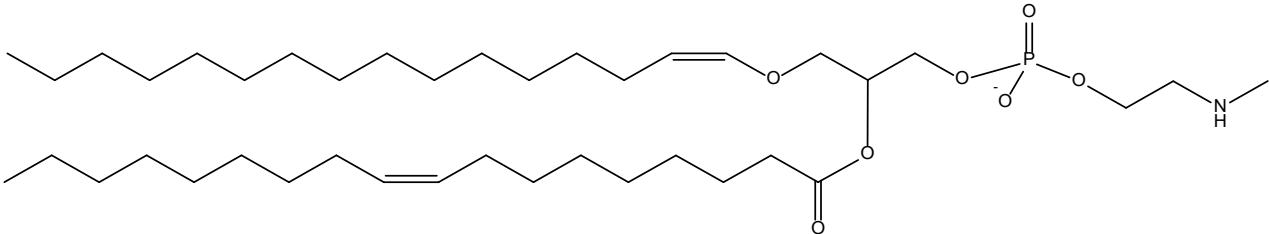
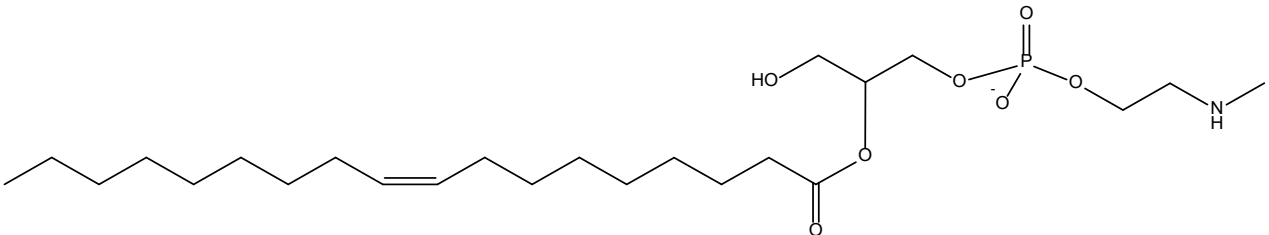
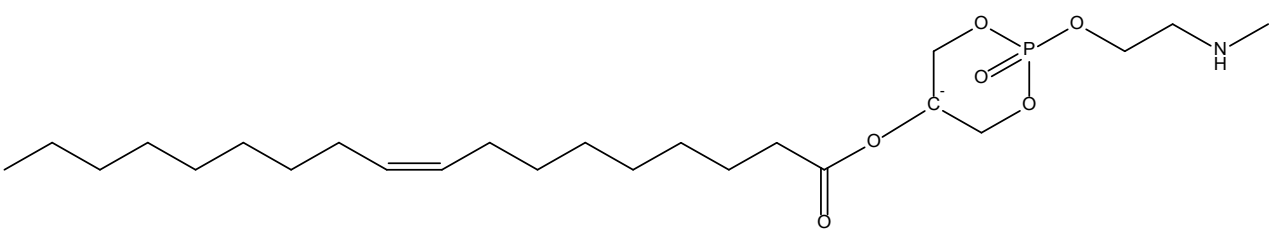
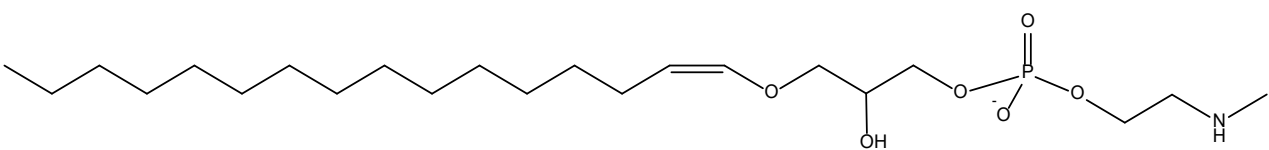
^C *Lucifera* and *Megasphaera* belongs to the same family, i.e. Veillonellaceae

^D *Salmonella* and *Raoultella* belong to the same family, i.e. Enterobacteriaceae

^E hypothetical protein, i.e. similar to phosphatidyl-*N*-methylethanolamine *N*-methyltransferase (*Arabidopsis thaliana*)
UniProtKB/Swiss-Prot:Q9SAH5

^F *Amphimedon queenslandica* and *Spongilla lacustris* belong to the same subclass Heteroscleromorpha (Class Demospongiae)

Table S2: Negative tandem MS of P-16:0/18:1/MMPE

Chemical Formula	Exact Mass	Relative abundance (%)	Structure
$[C_{40}H_{77}NO_7P]^-$	714.5445	24	
$[C_{24}H_{47}NO_7P]^-$	492.3098	5	
$[C_{24}H_{45}NO_6P]^-$	474.2992	4	
$[C_{22}H_{45}NO_6P]^-$	450.2993	18	

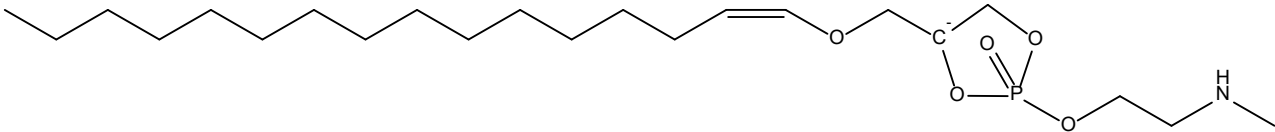
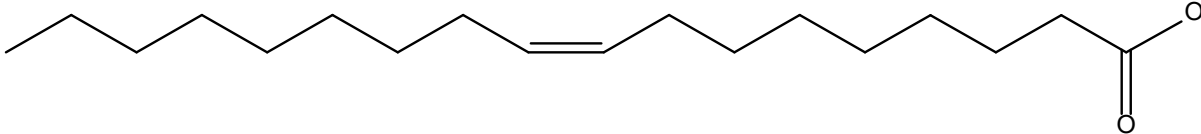
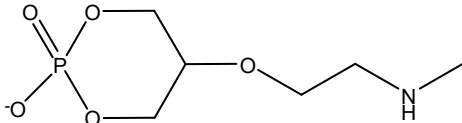
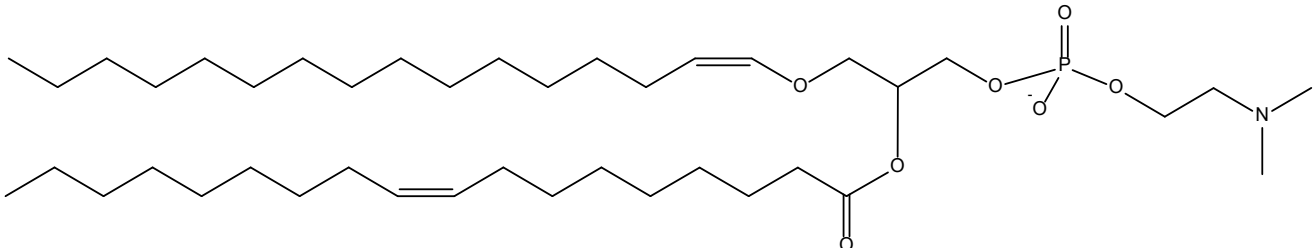
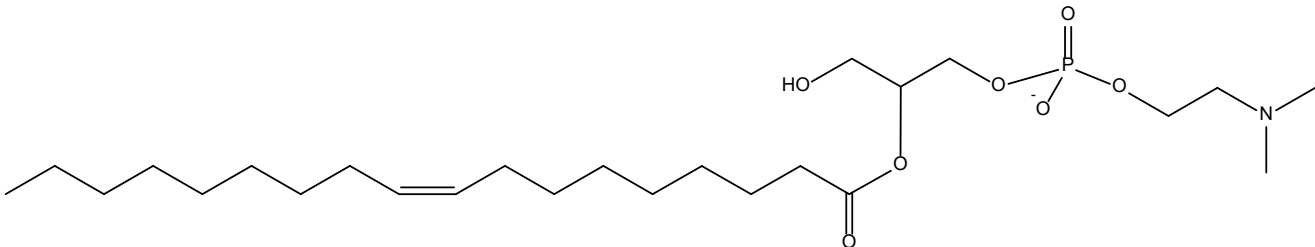
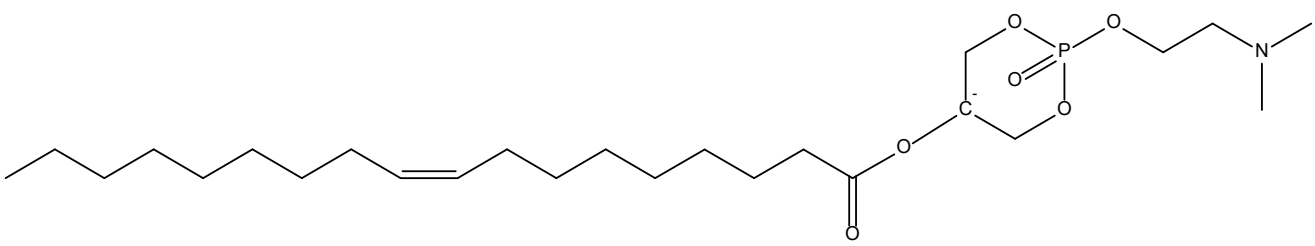
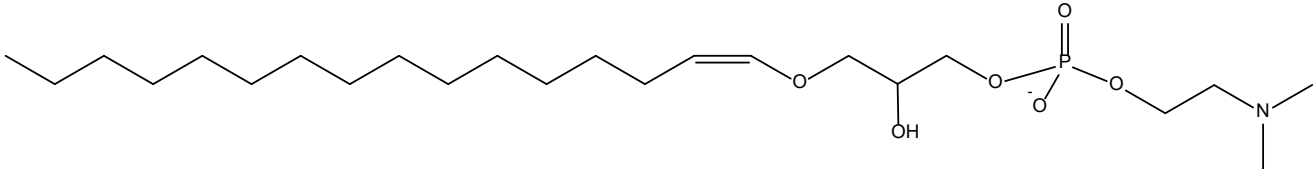
$[\text{C}_{22}\text{H}_{43}\text{NO}_5\text{P}]^-$	432.2886	6	
$[\text{C}_{18}\text{H}_{33}\text{O}_2]^-$	281.2488	100	
$[\text{C}_6\text{H}_{13}\text{NO}_5\text{P}]^-$	210.0539	25	

Table S3: Negative tandem MS of P-16:0/18:1/DMPE

Chemical Formula	Exact Mass	Relative abundance (%)	Structure
$[C_{41}H_{79}NO_7P]^-$	728.5602	21	
$[C_{25}H_{49}NO_7P]^-$	506.3255	4	
$[C_{25}H_{47}NO_6P]^-$	488.3147	4	
$[C_{23}H_{47}NO_6P]^-$	464.3148	19	

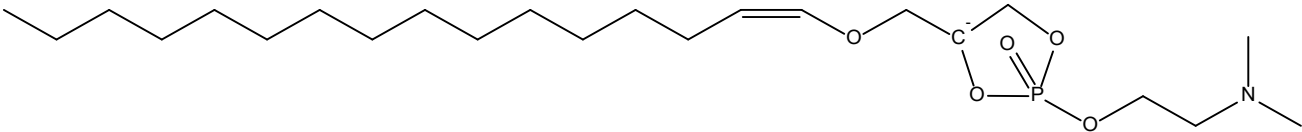
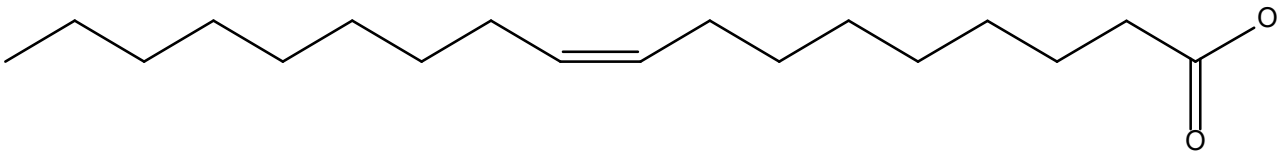
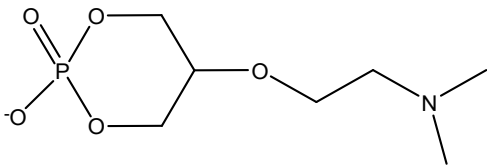
$[\text{C}_{23}\text{H}_{45}\text{NO}_5\text{P}]^-$	446.3044	5	
$[\text{C}_{18}\text{H}_{33}\text{O}_2]^-$	281.2488	100	
$[\text{C}_7\text{H}_{15}\text{NO}_5\text{P}]^-$	224.0694	22	

Table S4: RP-HPLC of alkenyl-acyl-acetyl glycerols from *Megasphaera cerevisiae*

plasmeynl	FA ₂	FA ₃	ACN ^a	DB	ECN	% ^b	% ^c	short	tr	[M+NH ₄] ⁺
16:1	16:1	2:0	34	2	30	24.1	1.84	34:2	14.75	608.5249
14:0	16:1	2:0	32	1	30	8.0	0.61	32:1	17.38	582.5092
17:1	16:1	2:0	35	2	31	19.2	1.46	35:2	25.47	622.5406
16:1	17:1	2:0	35	2	31	54.8	4.19	35:2	25.94	622.5406
14:0	17:1	2:0	33	1	31	19.2	1.47	33:1	28.55	596.5249
15:0	16:1	2:0	33	1	31	2.7	0.21	33:1	29.32	596.5249
18:1	16:1	2:0	36	2	32	37.4	2.86	36:2	36.13	636.5563
16:1	18:1	2:0	36	2	32	20.9	1.60	36:2	36.32	636.5563
17:1	17:1	2:0	36	2	32	43.9	3.35	36:2	37.13	636.5563
14:0	18:1	2:0	34	1	32	6.8	0.52	34:1	40.55	610.5406
16:1	16:0	2:0	34	1	32	30.5	2.33	34:1	42.02	610.5406
16:0	16:1	2:0	34	1	32	17.0	1.30	34:1	42.32	610.5406
15:0	17:1	2:0	34	1	32	7.6	0.58	34:1	42.63	610.5406
14:0	16:0	2:0	32	0	32	10.3	0.79	32:0	43.37	584.5249
19:1	16:1	2:0	37	2	33	44.6	3.40	37:2	47.29	650.5716
16:1	19:1	2:0	37	2	33	54.8	4.19	37:2	47.50	650.5716
18:1	17:1	2:0	37	2	33	84.3	6.43	37:2	48.32	650.5716
17:1	18:1	2:0	37	2	33	16.6	1.27	37:2	48.41	650.5716
15:0	18:1	2:0	35	1	33	2.2	0.17	35:1	51.74	624.5563
14:0	19:1	2:0	35	1	33	19.2	1.47	35:1	52.27	624.5563
17:1	16:0	2:0	35	1	33	24.3	1.85	35:1	52.73	624.5563
16:0	17:1	2:0	35	1	33	39.1	2.98	35:1	53.92	624.5563
15:0	16:0	2:0	33	0	33	3.7	0.29	33:0	54.96	598.5406
19:1	17:1	2:0	38	2	34	91.0	6.95	38:2	58.51	664.5873
17:1	19:1	2:0	38	2	34	43.9	3.35	38:2	58.82	664.5873
18:1	18:1	2:0	38	2	34	32.6	2.49	38:2	59.51	664.5873
15:0	19:1	2:0	36	1	34	7.6	0.58	36:1	62.10	638.5720
18:1	16:0	2:0	36	1	34	47.1	3.60	36:1	62.41	638.5720
18:0	16:1	2:0	36	1	34	4.0	0.30	36:1	62.98	638.5720
16:1	18:0	2:0	36	1	34	16.7	1.28	36:1	63.27	638.5720
16:0	18:1	2:0	36	1	34	14.7	1.12	36:1	63.76	638.5720
16:0	16:0	2:0	34	0	34	21.6	1.65	34:0	64.51	612.5563
14:0	18:0	2:0	34	0	34	5.3	0.40	34:0	65.57	612.5563
19:1	18:1	2:0	39	2	35	38.8	2.97	39:2	70.51	678.6031
18:1	19:1	2:0	39	2	35	84.3	6.43	39:2	70.71	678.6031
19:1	16:0	2:0	37	1	35	56.0	4.28	37:1	73.91	652.5873
16:0	19:1	2:0	37	1	35	39.1	2.98	37:1	74.12	652.5873
17:1	18:0	2:0	37	1	35	13.2	1.01	37:1	74.45	652.5873
18:0	17:1	2:0	37	1	35	10.3	0.79	37:1	75.16	652.5873

15:0	18:0	2:0	35	0	35	1.6	0.12	35:0	77.18	626.5720
19:1	19:1	2:0	40	2	36	100.0	7.64	40:2	81.89	692.6187
18:0	18:1	2:0	38	1	36	13.3	0.25	38:1	84.27	666.6030
18:1	18:0	2:0	38	1	36	26.1	2.00	38:1	85.31	666.6030
18:0	16:0	2:0	36	0	36	5.3	0.40	36:0	88.09	640.5877
16:0	18:0	2:0	36	0	36	11.7	0.89	36:0	88.54	640.5877
19:1	18:0	2:0	39	1	37	31.2	2.38	39:1	96.50	680.6187
18:0	19:1	2:0	39	1	37	10.3	0.79	39:1	96.57	680.6187
18:0	18:0	2:0	38	0	38	2.4	0.19	38:0	105.10	668.6187

^a Effective carbon number (ECN), double bond (DB), acyl carbon number (ACN), formula $ECN = ACN - (2 \times DB)$, retention time (tR), ^b 193
proportion of base peak (%), ^c proportion of total alkenyl-acyl-acetyl glycerols (%) 194

Table S5: RP-HPLC alkenyl-acyl-acetyl glycerols from *Pectinatus frisingensis*

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plasmeynl	FA ₂	FA ₃	ACN ^a	DB	ECN	% ^b	% ^c	short	t _R	[M+NH ₄] ⁺
15:1	17:1	2:0	34	2	30	24.7	3.29	34:2	14.02	608.5249
15:1	15:0	2:0	32	1	30	4.9	0.65	32:1	18.17	582.5092
15:1	18:1	2:0	35	2	31	22.9	3.06	35:2	25.11	622.5406
16:1	17:1	2:0	35	2	31	6.6	0.88	35:2	25.94	622.5406
14:0	17:1	2:0	33	1	31	11.1	1.48	33:1	28.55	596.5249
16:1	15:0	2:0	33	1	31	0.8	0.11	33:1	29.06	596.5249
14:0	15:0	2:0	31	0	31	1.9	0.25	31:0	32.78	570.5092
16:1	18:1	2:0	36	2	32	6.1	0.81	36:2	36.32	636.5563
17:1	17:1	2:0	36	2	32	89.2	11.90	36:2	37.13	636.5563
14:0	18:1	2:0	34	1	32	10.3	1.37	34:1	40.55	610.5406
15:1	17:0	2:0	34	1	32	9.8	1.31	34:1	41.59	610.5406
17:1	15:0	2:0	34	1	32	19.2	2.56	34:1	41.80	610.5406
15:0	17:1	2:0	34	1	32	100.0	13.33	34:1	42.63	610.5406
15:0	15:0	2:0	32	0	32	21.6	2.88	32:0	43.97	584.5249
18:1	17:1	2:0	37	2	33	23.6	3.14	37:2	48.32	650.5716
17:1	18:1	2:0	37	2	33	83.0	11.06	37:2	48.51	650.5716
15:0	18:1	2:0	35	1	33	93.0	12.40	35:1	51.74	624.5563
18:1	15:0	2:0	35	1	33	4.6	0.62	35:1	52.79	624.5563
16:1	17:0	2:0	35	1	33	2.3	0.30	35:1	53.10	624.5563
16:0	17:1	2:0	35	1	33	11.1	1.48	35:1	53.92	624.5563
14:0	17:0	2:0	33	0	33	4.2	0.56	33:0	54.67	598.5406
16:0	15:0	2:0	33	0	33	1.9	0.25	33:0	55.16	598.5406
18:1	18:1	2:0	38	2	34	21.9	2.92	38:2	59.51	664.5873
17:1	17:0	2:0	36	1	34	36.7	4.90	36:1	62.71	638.5720
17:0	17:1	2:0	36	1	34	21.3	2.84	36:1	62.93	638.5720
16:0	18:1	2:0	36	1	34	10.3	1.37	36:1	63.76	638.5720
17:0	15:0	2:0	34	0	34	4.1	0.55	34:0	66.15	612.5563
15:0	17:0	2:0	34	0	34	41.2	5.49	34:0	66.35	612.5563

17:0	18:1	2:0	37	1	35	19.8	2.63	37:1	74.12	652.5873
18:1	17:0	2:0	37	1	35	9.4	1.25	37:1	74.95	652.5873
18:0	17:1	2:0	37	1	35	8.3	1.10	37:1	75.16	652.5873
18:0	15:0	2:0	35	0	35	1.2	0.16	35:0	76.61	626.5720
16:0	17:0	2:0	35	0	35	4.2	0.56	35:0	77.54	626.5720
18:0	18:1	2:0	38	1	36	7.6	1.02	38:1	84.27	666.6030
17:0	17:0	2:0	36	0	36	8.4	1.12	36:0	88.73	640.5877
18:0	17:0	2:0	37	0	37	3.0	0.40	37:0	99.92	654.6030

Effective carbon number (ECN), double bond (DB), acyl carbon number (ACN), formula $ECN = ACN - (2 \times DB)$, retention time (tR), ^b 196
proportion of base peak (%), ^c proportion of total alkenyl-acyl-acetyl glycerols (%) 197

Table S6: Tandem MS alkenyl-acyl-acetyl glycerols

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Structure	P-16:0/18:1/2:0	P-18:0/18:1/2:0	P-15:1/17:0/2:0	P-17:1/15:0/2:0	P-17:1/17:1/2:0	P-16:1/18:1/2:0
Precursor ion $[M+NH_4]^+$	638.5718	666.6031	610.5407	610.5408	636.5563	636.5562
Precursor ion $[M+H]^+$	621.5452	649.5765	593.5142	593.5144	619.5300	619.5299
Neutral loss of <i>sn</i> -3 RCOOH + NH ₃ from $[M+NH_4]^+$	561.5241	589.5554	533.4936	533.4932	559.5088	559.5087
Neutral loss of <i>sn</i> -2 RCOOH + NH ₃ from $[M+NH_4]^+$	339.2894	367.3207	337.2745	337.2740	337.2740	339.2897
<i>sn</i> -2 acyl chain ($[RC=O +74]^+$)	339.2894	339.2894	327.2895	299.2583	325.2739	337.2741
<i>sn</i> -2 acyl chain ($[RC=O +74]^+$ with loss of H ₂ O)	321.2788	321.2788	309.2789	281.2477	307.2633	321.2790
<i>sn</i> -2 acyl chain ($[RC=O]^+$)	265.2526	265.2526	253.2528	225.2212	251.2371	265.2529
<i>sn</i> -2 acyl chain ($[RC=O]^+$ with loss of H ₂ O)	247.2420	247.2420	235.2419	207.2110	233.2266	247.2422

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Table S7: Tandem MS of both enantiomers P-16:0/18:1/2:0

Exact Mass	Relative abundance (%) First enantiomer	Relative abundance (%) Second enantiomer	Structure
638.5720	38	39	Precursor ion $[M+NH_4]^+$
621.5454	6	7	Precursor ion $[M+H]^+$
561.5244	100	100	Neutral loss of <i>sn</i> -3 RCOOH + NH ₃ from $[M+NH_4]^+$
339.2896	56	53	<i>sn</i> -2 acyl chain ($[RC=O+74]^+$) or Neutral loss of <i>sn</i> -2 RCOOH + NH ₃ from $[M+NH_4]^+$
321.2790	8	7	<i>sn</i> -2 acyl chain ($[RC=O+74]^+$ with loss of H ₂ O)
265.2528	16	15	<i>sn</i> -2 acyl chain ($[RC=O]^+$)
247.2422	6	7	<i>sn</i> -2 acyl chain ($[RC=O]^+$ with loss of H ₂ O)

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