Supplements

Radioactive Springs and Archaeal Life in Deep Groundwater Systems of Slovakia

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Table S1. Information about 21 springs, including their location, date of sampling and filtration together with filtered volume of water, number of used filters, temperature, flow rate, and radon activity concentration (RAC).

Sample[a]	GPS (°)	Sampling	Filtration	Filtered volume (mL)	No. of filters	Temperature (°C)	Flow rate (L/min)	RAC (Bq/L)
Dastinka	48.643743-17.9736137	10.6.2025 9:30	11.6.2025	3070	2	12.8	1.5	203.7 ± 8.2
Emily	48.3150278-17.2027778	17.6.2025 10:05	17.6.2025	3140	1	9.4	6.9	277.3 ± 16.1
Himligarka	48.2336881-17.1266469	18.6.2025 9:20	18.6.2025	3140	1	9.1	1.4	183.9 ± 7.8
Sielnica	48.861302-18.530103	7.6.2025 14:35	9.6.2025	3090	1	5.8	12.0	163.6 ± 9.5
Zbojnicka	48.2293806-17.1231061	18.6.2025 9:05	18.6.2025	3060	1	10.1	6.9	315.7 ± 18.4
Diery	49.25264-19.07216	13.6.2025 12:17	16.6.2025	3130	1	8.0	1.8	11.5 ± 1.5
Dolnanska	48.8485581-18.0836342	20.6.2025 10:45	23.6.2025	3200	2	12.5	0.8	85.1 ± 7.5
Frankovka	48.3281056-17.2053503	17.6.2025 10:33	17.6.2025	3060	1	9.2	18.0	13.4 ± 1.7
Chleb	49.1810019-19.0496897	12.6.2025 11:22	16.6.2025	3095	1	5.0	10.0	27.7 ± 2.6
Kada	49.0430119-19.677984	13.4.2025 12:28	14.-15.4.2025	3020	4	20.0	-	2.7 ± 0.7
Kostolany	48.41496896-18.2458056	13.5.2025 17:00	14.5.2025	2880	1	10.0	<15	14.2 ± 1.2
Patrovce	48.779336-18.091183	22.4.2025 12:00	23.4.2025	3050	1	8.8	6.0	33.8 ± 1.9
Bojnice	48.775388-18.571156	7.6.2025 16:00	9.6.2025	3110	1	35.6	0.8	3.5 ± 0.7
Kalameny	49.13337-19.41969	13.4.2025 11:43	15.4.2025	2700	2	33.0	-	9.0 ± 1.1
Kovacova	48.6079042-19.100739	3.5.2025 11:00	5.5.2025	3200	1	49.0	2.1	18.4 ± 1.1
Lucky	49.1342418-19.3972551	27.4.2025 13:06	29.4.2025	3090	1	35.2	-	13.1 ± 1.3
Piestany1	48.5869165-17.842682	10.6.2025 10:25	9.6.2025	3060	1	48.8	2.0	68.0 ± 4.6
Piestany2	48.586913-17.841779	10.6.2025 10:30	9.6.2025	3110	1	55.5	3.6	56.5 ± 3.7
Piestany3	48.5856106-17.8446239	10.6.2025 10:40	9.6.2025	3050	1	68.0	10.0	28.0 ± 2.2
Rajec	49.1292839-18.6821406	10.5.2025 13:58	12.5.2025	3060	1	35.9	2.0	4.5 ± 0.8
Teplice	48.909149-18.171703	16.5.2025 11:07	19.5.2025	2560	1	31.2	2.3	12.0 ± 1.5

[a] Spring name.
Yellowed name - radioactivity exceeding 100 Bq/L, i.e. recommended concentration according to WHO, i.e., Management of radioactivity in drinking-water (https://www.who.int/publications/i/item/9789241513746?utm_source=chatgpt.com).
Blue font, water temperature up to 20 ° C (cold spring),
Red font - water temperature above 30 ° C (temperate and warm springs).

Table S2. The distribution of individual archaeal lipids, comprising both core lipids and IPLs identified in samples from 21 springs.

Name	$[M+H]^+$	PI-AR	895.6998	diOH-GDGT	1334.3125	2Hex-P-GDGT-Uns(3)	1700.3477
AR	653.6812	PS-Ext-OH-AR	906.7521	PG-GDGT-Uns(3)	1450.2788	2Hex-P-GDGT-Uns(2)	1702.3633
OH-AR	669.6761	PI-Uns(2)-OH-AR	907.6634	PG-GDGT-Uns(2)	1452.2945	2Hex-P-GDGT-Uns(1)	1704.3790
2OH-AR	685.6711	PI-OH-AR	911.6947	Hex-GDGT-Uns(5*)	1454.2972	2Hex-P-GDGT	1706.3946
PA-AR	733.6470	PI-diOH-AR	927.6896	PG-GDGT-Uns(1)	1454.3101	PG-2Hex-GDGT-	1774.3845
PA-OH-AR	747.6273	PI-Ext-Uns(5)-OH-AR	971.6947	Hex-GDGT-Uns(4)	1456.3129	PG-2Hex-GDGT-	1776.4001
PE-AR	776.6892	2Hex-AR	977.7869	PG-GDGT	1456.3258	3Hex-GDGT-Uns(5*)	1778.4029
PME-AR	790.7048	PI-Ext-Uns(1)-OH-AR	979.7573	Hex-GDGT-Uns(3)	1458.3286	PG-2Hex-GDGT-	1778.4158
PE-OH-AR	792.6841	PI-Ext-OH-AR	981.7729	Hex-GDGT-Uns(2)	1460.3442	3Hex-GDGT-Uns(4)	1780.4185
DME-AR[a]	**804.7205**	PI-Ext-Uns(4)-diOH-	989.7053	Hex-GDGT-Uns(1)	1462.3599	PG-2Hex-GDGT	1780.4314
PME-OH-AR	806.6997	2Hex-Uns(1)-AR	992.7972	Hex-GDGT	1464.3756	3Hex-GDGT-Uns(3)	1782.4342
PG-AR	807.6837	2Hex-OH-AR	993.7817	Hex-P-GDGT-Uns(2)	1540.3105	3Hex-GDGT-Uns(2)	1784.4499
PS-Uns(5)-AR	810.6007	Hex-PS-OH-AR	998.7267	Hex-P-GDGT-Uns(1)	1542.3262	3Hex-GDGT-Uns(1)	1786.4655
Hex-AR	815.7341	3Hex-Uns(1)-AR	1154.8500	Hex-P-GDGT	1544.3417	3Hex-GDGT	1788.4812
PS-Uns(2)-AR	816.6477	3Hex-AR	1156.8656	diPG-GDGT-Uns(3)	1604.2819	3Hex-P-GDGT-	1858.3695
PG-Uns(2)-OH-AR	819.6473	GDGT-Uns(5*)	1292.2444	diPG-GDGT-Uns(2)	1606.2976	3Hex-P-GDGT-Uns(3)	1862.4007
PS-AR	820.6790	GDGT-Uns(3)	1296.2757	diPG-GDGT-Uns(1)	1608.3132	3Hex-P-GDGT-Uns(2)	1864.4163
DME-OH-AR	**820.7154**	GDGT-Uns(2)	1298.2914	diPG-GDGT	1610.3289	3Hex-P-GDGT-Uns(1)	1866.4319
PG-OH-AR	823.6786	GDGT-Uns(1)	1300.3070	2Hex-GDGT-Uns(5*)	1616.3507	3Hex-P-GDGT	1868.4474
PS-Uns(2)-OH-AR	832.6426	GDGT	1302.3227	2Hex-GDGT-Uns(4)	1618.3658	4Hex-GDGT-Uns(5*)	1940.4560
PS-OH-AR	836.6739	OH-GDGT-Uns(5*)	1308.2393	2Hex-GDGT-Uns(3)	1620.3815	4Hex-GDGT-Uns(4)	1942.4716
PG-diOH-AR	839.6736	OH-GDGT-Uns(3)	1312.2706	2Hex-GDGT-Uns(2)	1622.3971	4Hex-GDGT-Uns(3)	1944.4872
Hex-OH-AR	848.7549	OH-GDGT-Uns(2)	1314.2863	2Hex-GDGT-Uns(1)	1624.4127	4Hex-GDGT-Uns(2)	1946.5027
PS-Uns(1)-diOH-AR	850.6532	OH-GDGT-Uns(1)	1316.3019	2Hex-GDGT	1626.4283	4Hex-GDGT-Uns(1)	1948.5184
PS-diOH-AR	852.6688	OH-GDGT	1318.3176	2Hex-P-GDGT-	1696.3166	4Hex-GDGT	1950.5340
PG-Uns(1)-Ext-OH-AR	891.7412	diOH-GDGT-Uns(5*)	1324.2342	2Hex-P-GDGT-Uns(4)	1698.3322		

[a] **bold**, previously undescribed molecular species, both in nature and synthesized in the laboratory.

Table S3. The content of individual molecular species of core lipids in ng/L in 21 springs, determined by shotgun lipidomic.

Spring[a]	AR[b]	OH-AR	2OH-AR	GDGT	OH-GDGT	diOH-GDGT
Dastinka	44.8	22.7	29.2	434.0	374.5	392.0
Emily	65.6	56.5	47.4	490.0	465.5	441.0
Himligarka	51.3	31.8	43.5	451.5	399.0	430.5
Sielnica	40.9	51.3	34.4	423.5	451.5	406.0
Zbojnicka	57.8	53.9	59.1	469.0	458.5	472.5
Diery	155.9	166.1	104.9	297.5	318.5	192.5
Dolnanska	150.8	118.5	132.1	287.0	220.5	248.5
Frankovka	193.3	94.7	89.6	374.5	171.5	161.0
Chleb	118.5	96.4	103.2	220.5	175.0	189.0
Kada	174.6	69.2	84.5	336.0	119.0	150.5
Kostolany	196.7	123.6	99.8	381.5	231.0	182.0
Patrovce	111.7	101.5	89.6	206.5	185.5	161.0
Bojnice	266.1	287.1	278.7	22.4	36.4	30.8
Kalameny	272.4	276.6	303.9	26.6	29.4	47.6
Kovacova	272.4	282.9	270.3	26.6	33.6	25.2
Lucky	285.0	320.7	295.5	35.0	58.8	42.0
Piestany 1	293.4	293.4	310.2	40.6	40.6	51.8
Piestany 2	335.4	364.8	322.8	68.6	88.2	60.2
Piestany 3	266.1	287.1	278.7	22.4	36.4	30.8
Rajec	272.4	276.6	303.9	26.6	29.4	47.6
Teplice	272.4	282.9	270.3	26.6	33.6	25.2

[a] Spring name in:

Blue font, water temperature up to 20 ° C (cold spring),

Red font - water temperature above 30 °C (temperate and warm springs),

Yellowed name - radioactivity exceeding 100 Bq/L, i.e. recommended concentration according to WHO.

[b] Abbreviations: AR - archaeol (C20-C20 isoprenoidal chains); OH-AR - monohydroxylated-archaeol; 2OH-AR - dihydroxylated-archaeol; glycerol dialkyl glycerol tetraether - GDGT; monohydroxylated glycerol dialkyl glycerol tetraether - OH-GDGT; diOH-GDGT - dihydroxylated glycerol dialkyl glycerol tetraether.

Table S4. The content of individual molecular species of glycosides in ng/L in 21 springs, determined by shotgun lipidomic.

Spring[a]	Hex-AR[a]	2Hex-AR	3Hex-AR	Hex-GDGT	2Hex-GDGT	3Hex-GDGT	4Hex-GDGT
Dastinka	6.5	7.7	7.2	271.3	254.3	330.5	197.9
Emily	3.8	7.1	8.5	297.6	333.0	274.0	245.9
Himligarka	10.2	11.7	9.5	296.7	278.1	299.5	210.1
Sielnica	7.9	7.9	8.9	265.8	321.8	288.4	242.6
Zbojnicka	13.0	7.5	6.7	300.9	343.5	316.7	251.4
Diery	114.2	92.6	128.0	191.3	181.4	144.4	185.5
Dolnanska	112.1	130.1	125.1	197.9	245.9	251.4	186.8
Frankovka	108.9	136.0	122.3	116.6	129.6	160.3	112.7
Chleb	106.0	90.6	110.7	162.9	150.0	109.9	120.1
Kada	121.6	98.3	130.4	109.5	111.9	123.7	125.6
Kostolany	98.5	111.1	117.0	124.9	130.8	140.2	155.8
Patrovce	106.3	100.1	123.9	252.7	230.8	228.1	174.2
Bojnice	165.8	187.8	168.8	15.8	15.0	11.6	21.3
Kalameny	167.9	144.2	146.3	9.0	7.6	3.4	14.3
Kovacova	186.5	195.0	120.7	16.2	15.5	12.1	16.9
Lucky	174.1	187.6	152.7	9.9	8.6	4.5	11.5
Piestany1	197.7	168.9	156.0	6.5	7.7	7.2	6.9
Piestany 2	170.6	144.6	150.2	9.9	7.1	8.5	19.5
Piestany3	216.0	208.4	174.2	6.9	7.5	6.7	9.9
Rajec	148.1	133.7	210.1	7.6	9.5	8.0	8.9
Teplice	219.9	212.8	178.9	12.8	18.7	15.1	15.6

[a,b] Abbreviations: Hex-AR - glycosyl (hexosyl) archaeol; 2Hex-AR - diglycosyl (dihexosyl) archaeol; 3Hex-AR - triglycosyl (trihexosyl) archaeol; Hex-GDGT - glycosyl (hexosyl) glycerol dialkyl glycerol tetraether; 2Hex-GDGT - diglycosyl (dihexosyl) glycerol dialkyl glycerol tetraether; 3Hex-GDGT - triglycosyl (trihexosyl) glycerol dialkyl glycerol tetraether; 4Hex-GDGT - tetraglycosyl (tetrahexosyl) glycerol dialkyl glycerol tetraether.

Table S5. The content of individual molecular species of archaeol phospholipids in ng/L in 21 springs, determined by shotgun lipidomic.

Spring[a]	PA-AR[b]	PE-AR	PME-AR	DME-AR[c]	PG-AR	PS-AR	PI-AR
Dastinka	164.2	171.4	108.5		221.7	286.3	173.9
Emily	153.7	165.2	135.6		288.4	238.4	214.6
Himligarka	148.5	128.4	130.2		241.9	260.0	184.2
Sielnica	146.7	165.3	149.2	231.4	278.9	250.6	211.8
Zbojnicka	173.7	149.3	138.4		297.3	274.6	219.2
Diery	96.3	91.0	111.2		186.5	157.8	163.4
Dolnanska	150.8	128.7	133.5		201.8	199.5	164.5
Frankovka	101.2	116.4	112.2		159.9	128.6	101.7
Chleb	103.0	84.7	114.7	144.2	127.8	93.3	108.0
Kada	98.5	121.4	109.8		133.3	99.3	112.6
Kostolany	109.2	89.5	116.7		182.8	153.8	138.2
Patrovce	89.7	100.3	105.3		119.5	84.2	153.8
Bojnice	67.3	54.9	60.0		12.7	12.3	24.2
Kalameny	36.7	42.9	51.2		12.2	13.4	18.3
Kovacova	68.8	50.4	8.1		12.5	11.9	20.5
Lucky	63.4	44.5	28.0		14.2	13.0	15.9
Piestany 1	52.3	32.3	14.6		12.9	13.7	12.0
Piestany 2	73.0	55.0	39.6		16.1	14.2	22.7
Piestany 3	46.6	25.9	7.6		12.7	12.3	14.6
Rajec	74.5	56.7	41.5		12.2	13.4	13.7
Teplice	50.0	29.7	11.8		12.5	11.9	19.4

[a,b] Abbreviations: PA-AR - phosphatidic acid archaeol; PE-AR - phosphatidylethanolamine archaeol; PME-AR - phosphatidylmethylethanolamine archaeol; DME-AR - phosphatidyldimethylethanolamine archaeol; PG-AR - phosphatidylglycerol archaeol; PS-AR - phosphatidylserine archaeol; PI-AR - phosphatidylinositol archaeol.
[c] Identified in only two springs; see text.

Table S6. Results of the analysis of the KEGG database (https://www.genome.jp/kegg/pathway.html) showing that more than 30 archaeal species are putatively contained by two key *N*-methyltransferases (EC 2.1.1.17 and EC 2.1.1.71).

Kegg code	Genus	Species	Strain
hje	*Halalkalicoccus*	*jeotgali*	
harc	*Halococcoides*	*cellulosivorans*	
haln	*Halolamina*	sp.	CBA1230
hah	*Halophilic*	*archaeon*	
hxa	*Halopiger*	*xanaduensis*	
hwc	*Haloquadratum*	*walsbyi*	C23
hezz	*Halorubrum*	*ezzemoulense*	
hss	*Halorubrum*	*ruber*	
halb	*Halorubrum*	sp.	BOL3-1
hlt	*Halosimplex*	*litoreum*	
hpel	*Halosimplex*	*pelagicum*	
hrr	*Halosimplex*	*rubrum*	
hlr	*Halostagnicola*	*larsenii*	
hakz	*Haloterrigena*	*alkaliphila*	
hsal	*Haloterrigena*	*salifodinae*	
htu	*Haloterrigena*	*turkmenica*	
mefw	*Methanochimaera*	*problematica*	
naj	*Natrarchaeobaculum*	*aegyptiacum*	
nag	*Natrarchaeobaculum*	*sulfurireducens*	AArc-Mg
nan	*Natrarchaeobaculum*	*sulfurireducens*	AArc1
nmg	*Natrialba*	*magadii*	
haly	*Natrinema*	*halophilum*	
hlo	*Natrinema*	*longum*	
npe	*Natrinema*	*pellirubrum*	
hjt	*Natrinema*	*thermotolerans*	
nvr	*Natrinema*	*versiforme*	
nge	*Natronobacterium*	*gregoryi*	
hlc	*Natronobacterium*	*lacisalsi*	
nou	*Natronococcus*	*occultus*	
nho	*Natronomonas*	*halophila*	
nas	*Natronorubrum*	*aibiense*	
nbg	*Natronorubrum*	*bangense*	
hda	*Natronorubrum*	*daqingense*	
sali	*Salinarchaeum*	sp.	Harcht-Bsk1

Table S7. Variable Importance in Projection (VIP) scores and PLS loading values for lipids along the radon-activity and temperature gradients derived from the first two PLS components.

Lipid Compound	*VIP score*	*PLS loading – radon gradient*	*PLS loading – temperature gradient*
AR	0.876	-0.197	-0.114
OH-AR	1.109	-0.193	-0.436
2OH-AR	1.180	-0.185	-0.478
GDGT	0.906	0.355	0.270
OH-GDGT	0.972	0.372	-0.290
diOH-GDGT	1.141	0.386	-0.451
Hex-AR	0.942	-0.160	0.048
2Hex-AR	0.963	-0.156	0.092
3Hex-AR	1.297	-0.156	0.278
Hex-GDGT	0.900	0.250	0.058
2Hex-GDGT	0.924	0.277	-0.026
3Hex-GDGT	0.875	0.267	-0.015
4Hex-GDGT	0.887	0.188	0.143
PA-AR	0.955	0.095	-0.061
PE-AR	0.866	0.099	0.017
PME-AR	1.322	0.078	0.255
PG-AR	0.858	0.227	0.057
PS-AR	0.948	0.231	-0.096
PI-AR	0.887	0.160	0.122

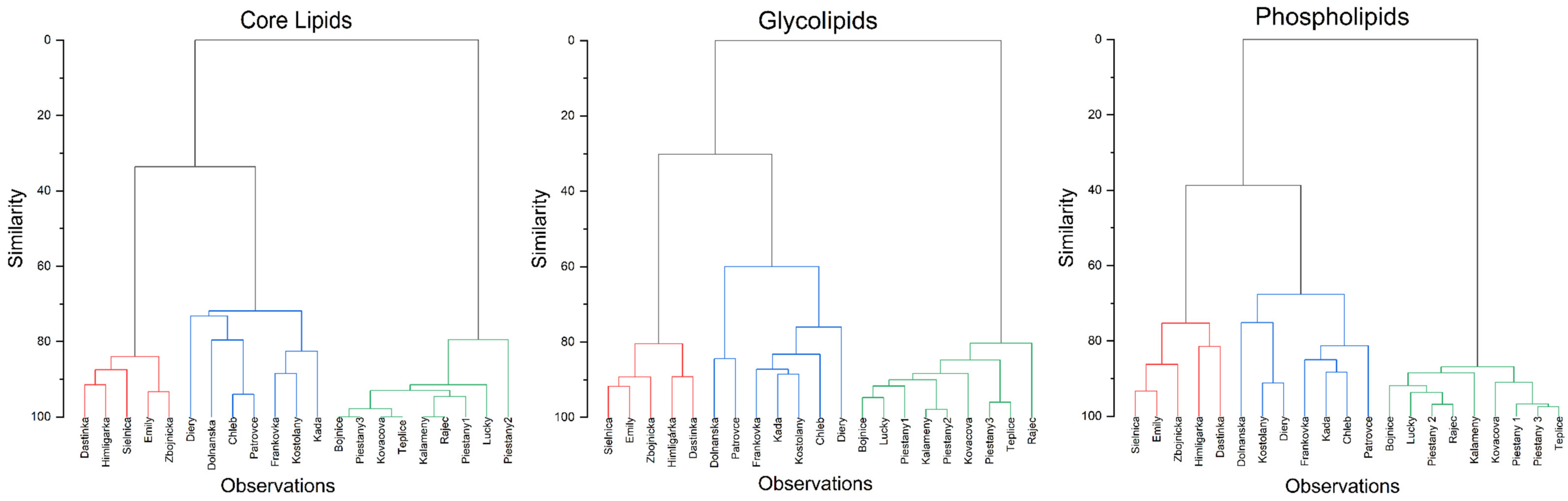

Fig. S1. Hierarchical cluster analysis (HCA) dendrograms of core lipids, glycolipids, and phospholipids, indicating three main clusters of springs.

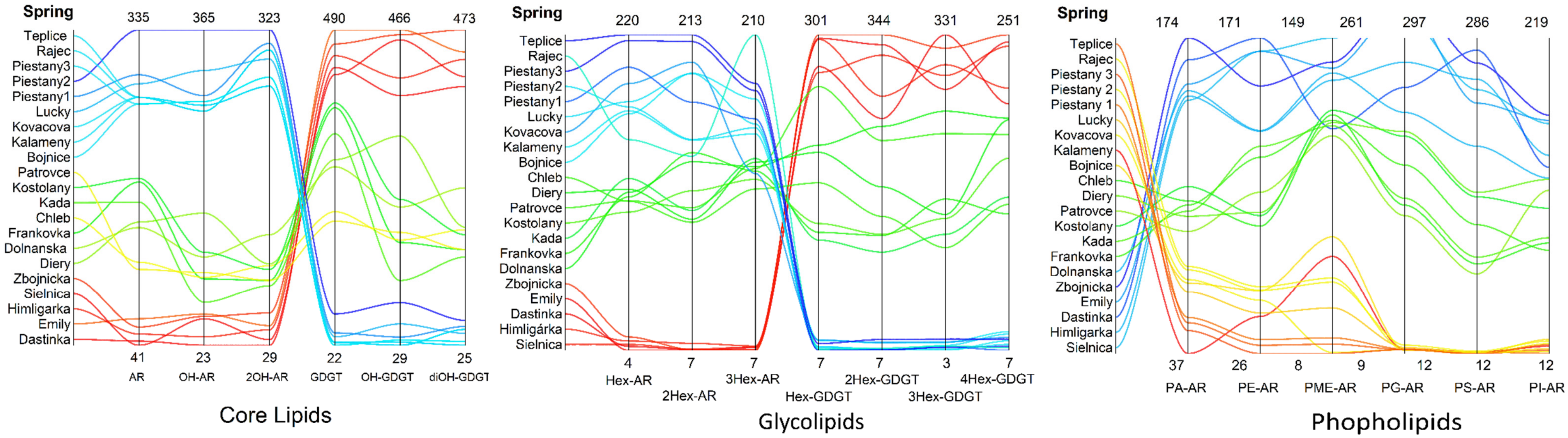

Fig. S2. Relative abundances of individual archaeal lipid biomarkers in the investigated springs, grouped by lipid class. Springs with similar lipid profiles may appear in similar shades, which can visually resemble sample grouping.

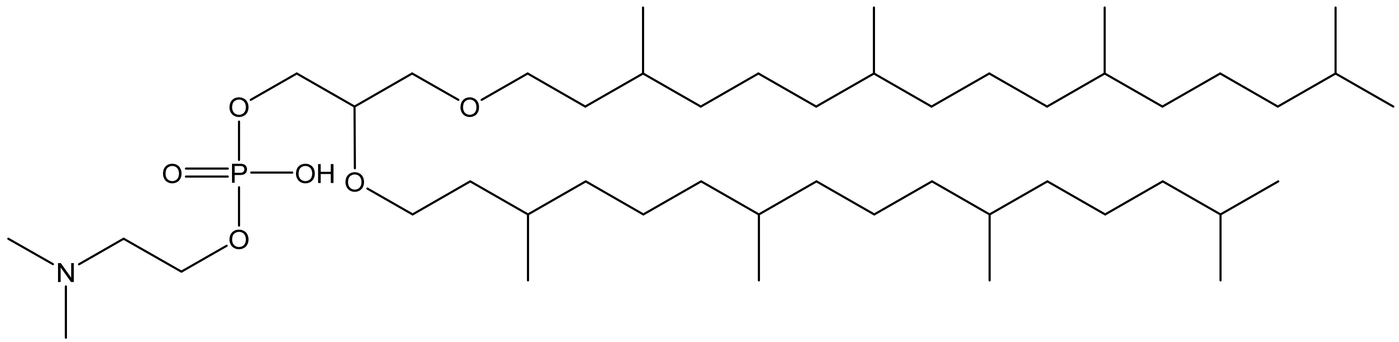

Fig. S3. Structure of DMPE-AR, i.e., 2,3-bis((3,7,11,15-tetramethylhexadecyl)oxy)propyl (2-(dimethylamino)ethyl) hydrogen phosphate.

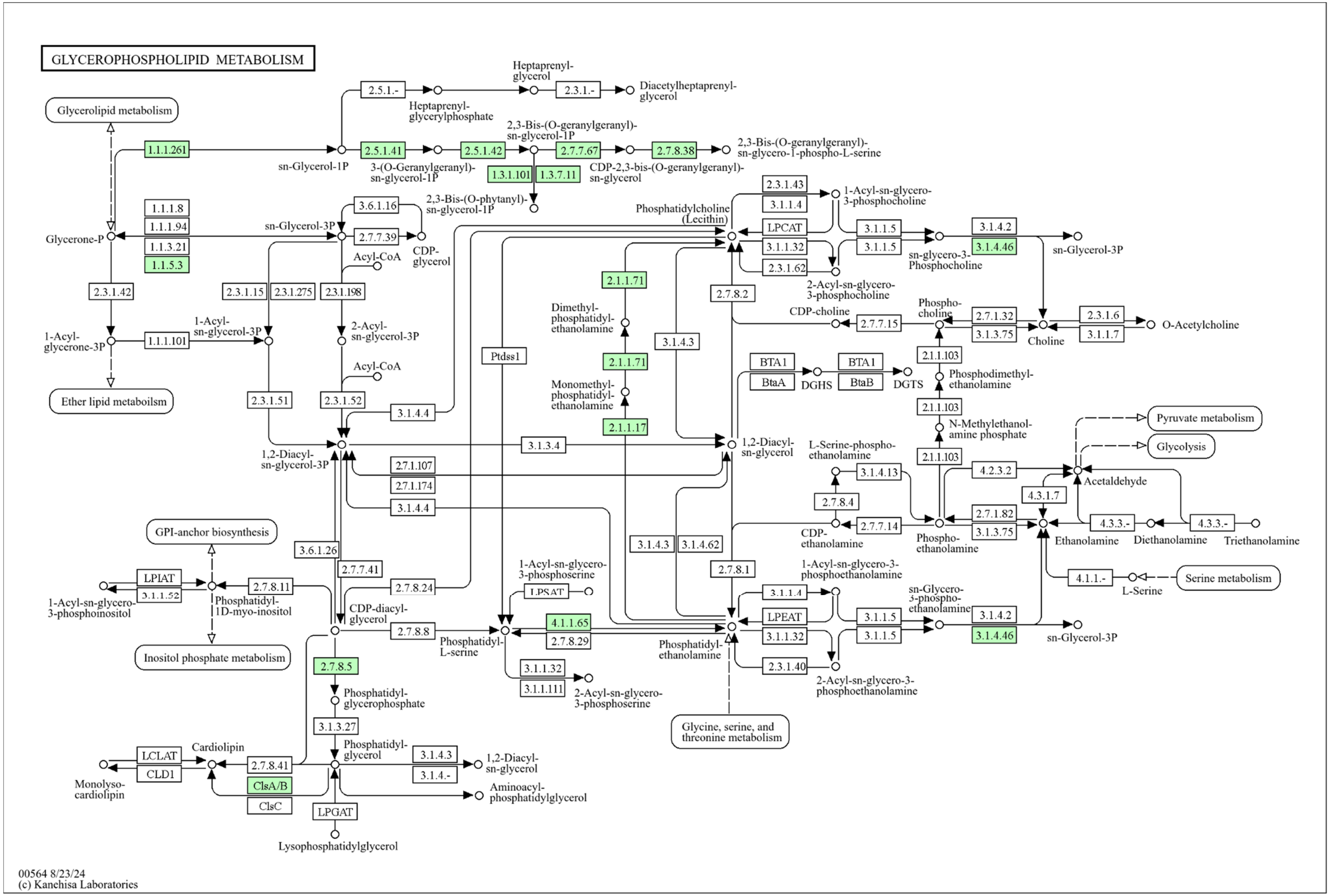

Fig. S4. Kegg *Haloquadratum walsbyi*. DSM 16790 ano

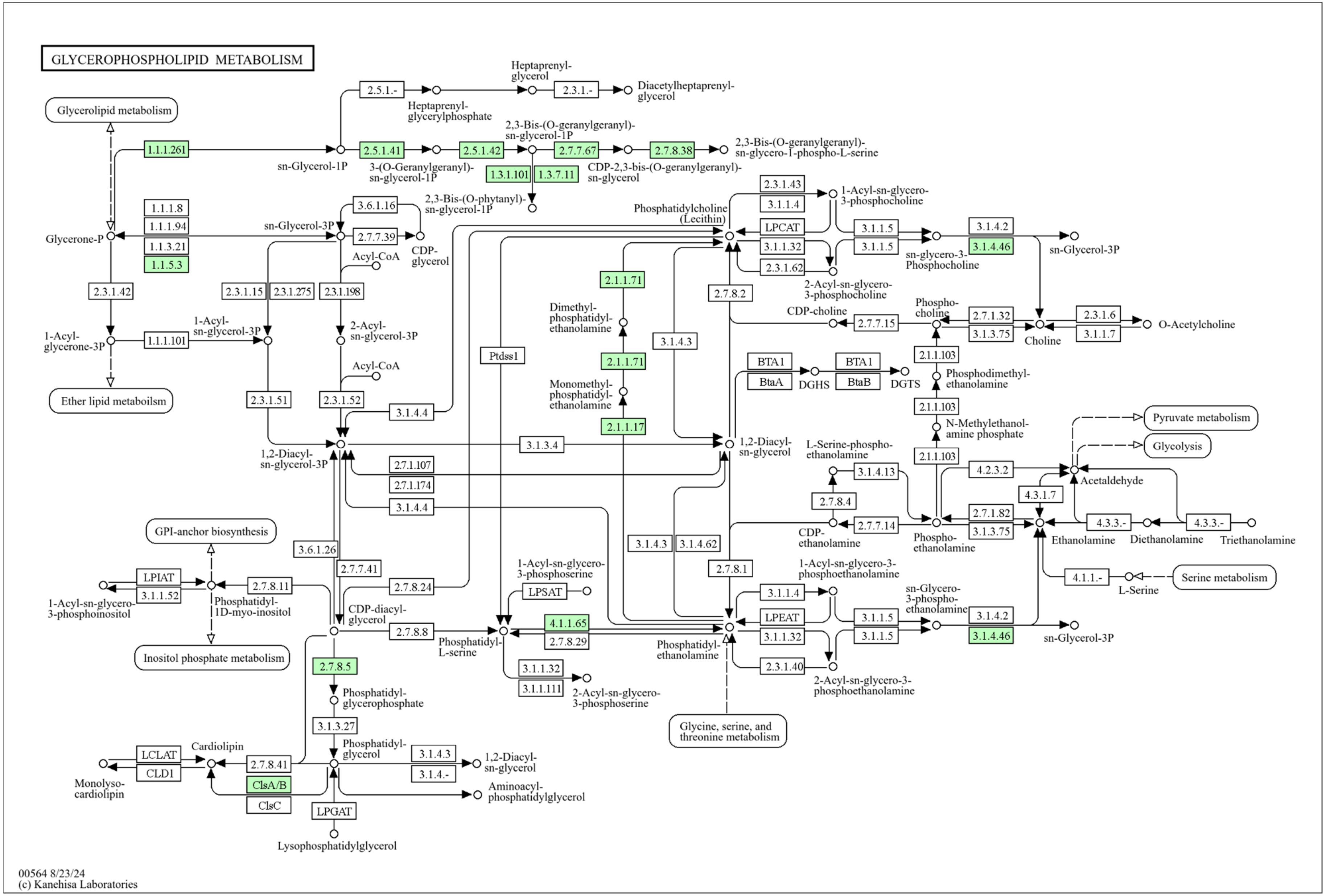

Fig. S5. Kegg *Natrarchaeobaculum sulfurireducens*. AArc1 (strain) ano

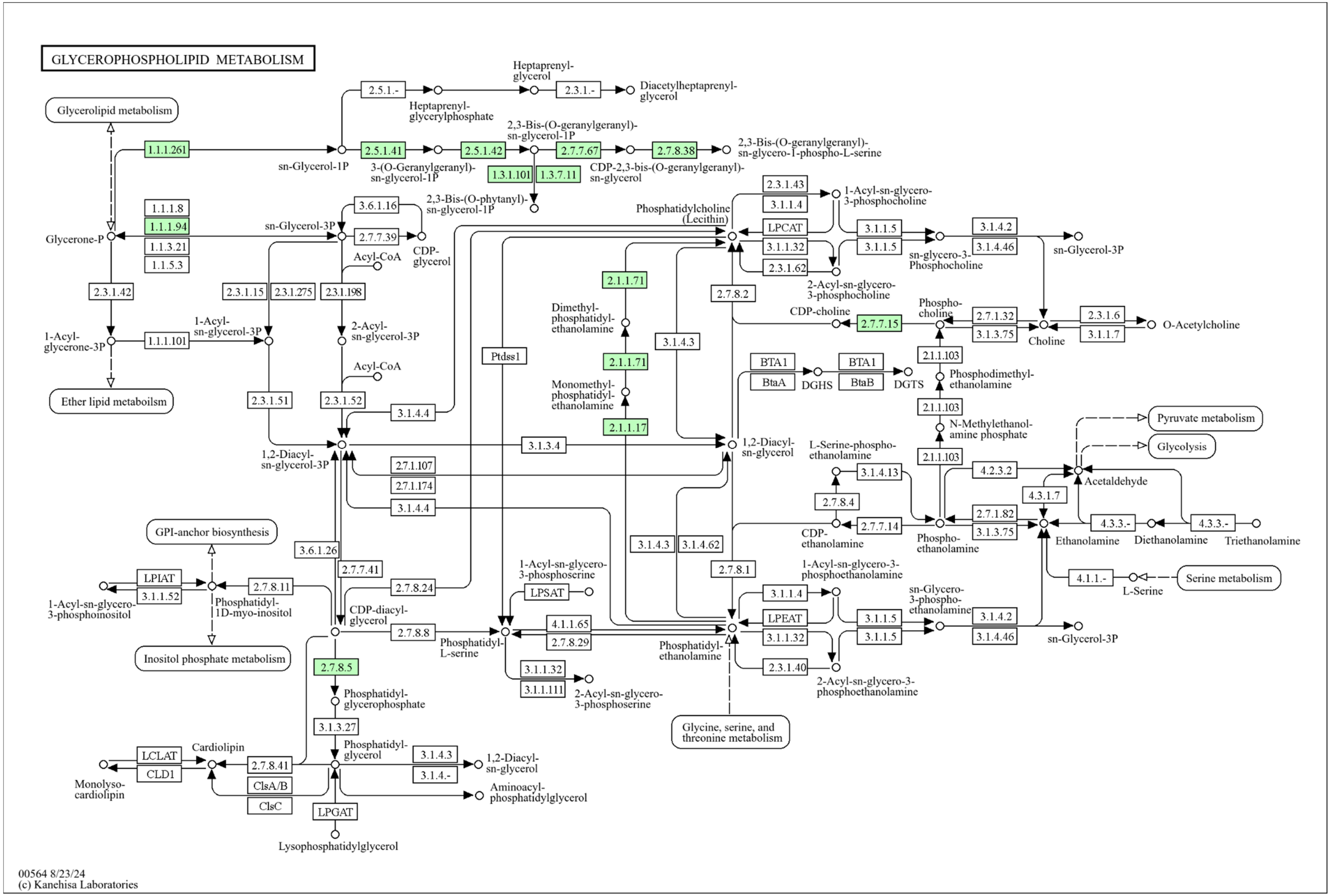

Fig. S6. Kegg *Methanobrevibacter smithii*. DSM 11975 ano