Supporting Information

Stereochemistry of phosphatidylglycerols from thermotolerant bacteria isolated thermal springs

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Table 1S. Biomass yields, total extracted lipids, and phosphatidylglycerols from seven strains of bacteria cultivated at different temperatures.

Name of bacteria	Isolate	strain	temp (°C)	biomass (mg)[a]	total lipids of lyophilized biomass (mg)	% PG of total lipids[b]
Bacillus cereus	1_50L8_P3	P23	44	54	1.17	1.04 ± 0.07
Bacillus cereus	1_50L8_P3	P23	12	16	0.60	0.92 ± 0.01
Bacillus licheniformis	AFA_V2	V2	50	54	1.48	1.14 ± 0.06
Bacillus licheniformis	AFA_V2	V2	20	15	0.99	1.84 ± 0.07
Bacillus subtilis subsp. *inaquosorum*	57_81	S8	50	22	0.41	1.23 ± 0.04
Bacillus subtilis subsp. *inaquosorum*	57_81	S8	15	70	2.42	1.19 ± 0.06
Bacillus tequilensis	1_1L8_M6	M10	50	26	0.76	0.75 ± 0.05
Bacillus tequilensis	1_1L8_M6	M10	15	55	2.10	0.91 ± 0.07
Geobacillus stearothermophilus	38	Pl	60	29	0.97	1.42 ± 0.08
Geobacillus stearothermophilus	38	Pl	46	48	2.43	1.54 ± 0.05
Paenibacillus favisporus	1_5L8_P5	P22	46	34	0.91	0.98 ± 0.08
Paenibacillus favisporus	1_5L8_P5	P22	17	100	2.05	0.87 ± 0.10
Priestia aryabhattai	1_2L8_Ml	M7	46	50	1.57	1.12 ± 0.07
Priestia aryabhattai	1_2L8_Ml	M7	14	54	2.35	1.14 ± 0.08

[a] Data are presented as arithmetic means from two cultivations.

[b] Data of technical replicates (six chromatographic analyses) ± standard deviations (S.D.).

Table 2S. Product ions for 15:0/15:0-PG obtained using tandem MS.

m/z	Ion Description	Natural PG	Standard PG
152.9959	Glycerol-3-phosphate ion with loss of H_2O	9	9
209.0222	Glycerophosphoglycerol ion $-2H_2O$	5	6
227.0327	Glycerophosphoglycerol ion $-H_2O$	7	7
241.2174	$RCOO^-$ ion	100	100
245.0433	Glycerophosphoglycerol ion	5	5
377.2100	Neutral loss of the RCOOH group and glycerol from $[M-H]^-$	17	19
395.2205	Loss of acyl chain as ketene (RCH=C=O) and glycerol from $[M-H]^-$	12	11
451.2468	Neutral loss of the RCOOH group from $[M-H]^-$	20	21
469.2574	Loss of acyl chain as ketene (RCH=C=O) from $[M-H]^-$	16	17
619.4346	Loss of glycerol from the precursor ion	16	16
693.4714	Precursor ion $[M-H]^-$	60	55

Table 3S. Tandem MS of DNPU-16:0/18:1-PG.

m/z	Abundance (relative %)	Ion Description
1165.5327	100	$[M-H]^-$
956.5254	45	$[M-H-DNPU]^-$
747.5182	22	$[M+H-2xDNPU]^-$
509.2885	3	Loss of *sn*-2 acyl chain as ketene (RCH=C=O) from $[M+H-2xDNPU]^-$
491.2779	5	Loss of the *sn*-1 acyl chain as ketene (RCH=C=O) from $[M+H-2xDNPU]^-$
483.2729	8	Neutral loss of the *sn*-2 RCOOH group from $[M+H-2xDNPU]^-$
465.2623	6	Neutral loss of the *sn*-1 RCOOH group from $[M+H-2xDNPU]^-$
435.2517	3	Loss of *sn*-2 acyl chain as ketene (RCH=C=O) and glycerol from $[M+H-2xDNPU]^-$
417.2412	5	Loss of the *sn*-1 acyl chain as ketene (RCH=C=O) and glycerol from $[M+H-2xDNPU]^-$
409.2361	3	Neutral loss of the *sn*-2 RCOOH group and glycerol from $[M+H-2xDNPU]^-$
391.2255	9	Neutral loss of the *sn*-1 RCOOH group and glycerol from $[M+H-2xDNPU]^-$
281.2486	23	*sn*-2 $RCOO^-$ ion
255.2331	11	*sn*-1 $RCOO^-$ ion
245.0432	3	Glycerophosphoglycerol ion
227.0326	5	Glycerophosphoglycerol ion $-H_2O$
209.0221	3	Glycerophosphoglycerol ion $-2H_2O$
152.9958	6	Glycerol-3-phosphate ion with loss of H_2O

Table 4S. Tandem MS spectrum of non-derivatized 16:0/18:1-PG

m/z	Abundance (relative %)	Ion Description
747.5182	35	Precursor ion $[M-H]^-$
673.4814	18	Loss of glycerol from the precursor ion
509.2885	5	Loss of sn1 acyl chain as ketene (RCH=C=O) from $[M-H]^-$
491.2779	7	Neutral loss of the *sn*-1 RCOOH group from $[M-H]^-$
483.2729	13	Loss of the *sn*-2 acyl chain as ketene (RCH=C=O) from $[M-H]^-$
465.2623	9	Neutral loss of the *sn*-2 RCOOH group from $[M-H]^-$
435.2517	4	Loss of *sn*-1 acyl chain as ketene (RCH=C=O) and glycerol of $[M-H]^-$
417.2412	6	Neutral loss of the *sn*-1 RCOOH group and glycerol from $[M-H]^-$
409.2361	3	Loss of the *sn*-2 acyl chain as ketene (RCH=C=O) and glycerol from $[M-H]^-$
391.2255	16	Neutral loss of the *sn*-2 RCOOH group and glycerol from $[M-H]^-$
281.2486	100	*sn*-2 $RCOO^-$ ion
255.2330	42	*sn*-1 $RCOO^-$ ion
245.0432	4	Glycerophosphoglycerol ion
227.0326	6	Glycerophosphoglycerol ion $-H_2O$
209.0221	5	Glycerophosphoglycerol ion $-2H_2O$
152.9958	8	Glycerol-3-phosphate ion with loss of H_2O

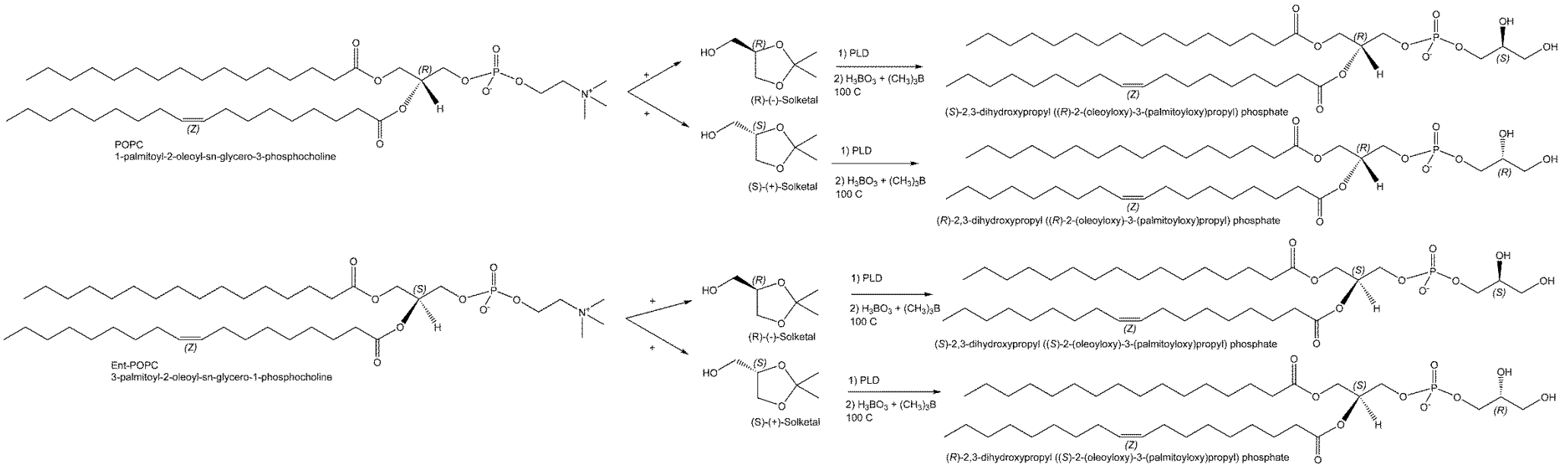

Figure 1S. Preparation of four isomers of 16:0/18:1-PG (*R*,*R*, *R*,*S*, *S*,*R,* and *S*,*S*) from two enantiomers of PC (*R* or *S*) and two enantiomers of two solketals (*R* or *S*).

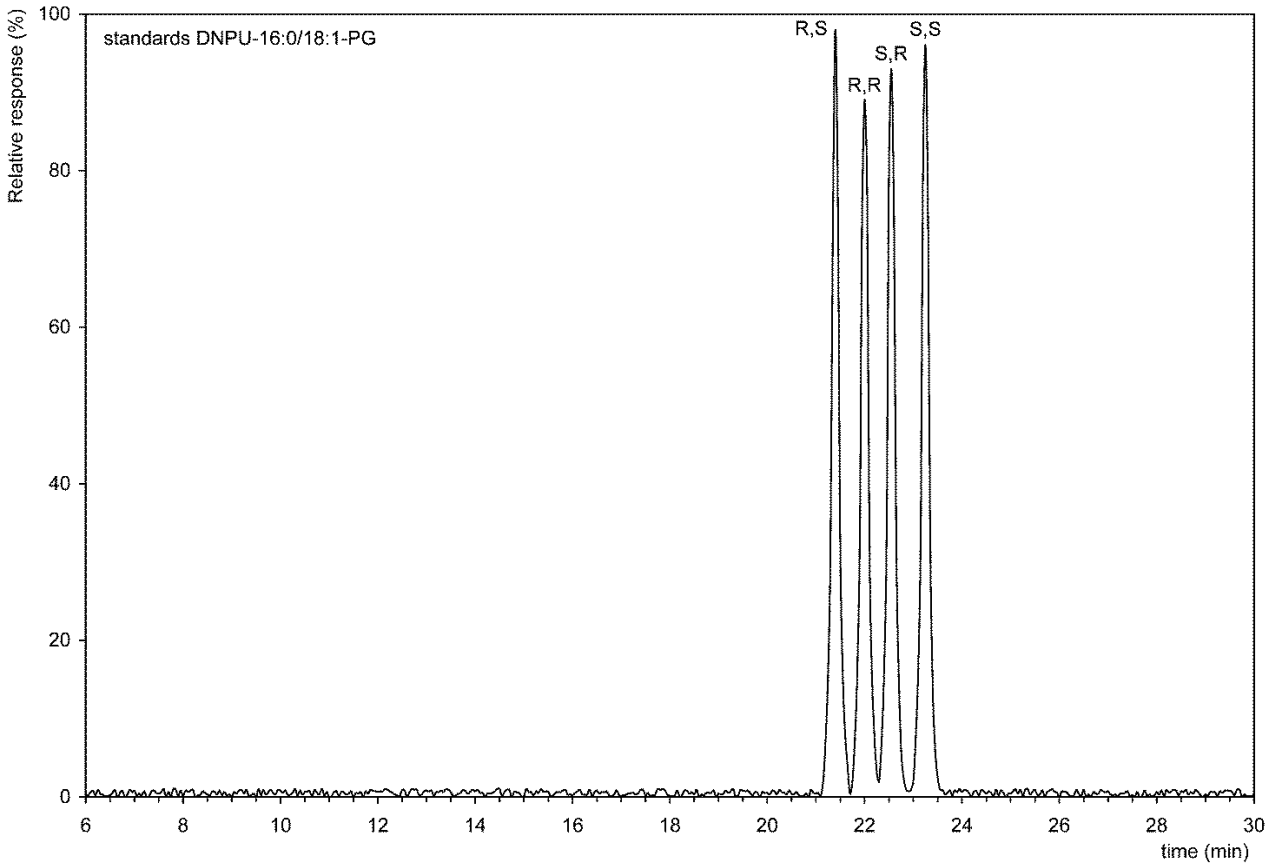

Figure 2S. The LC-MS separation of the four isomers of 16:0/18:1-PG (*R,R, R,S, S,R*, and *S,S*) as bis(3,5-dinitrophenyl) urethanes on a chiral column.

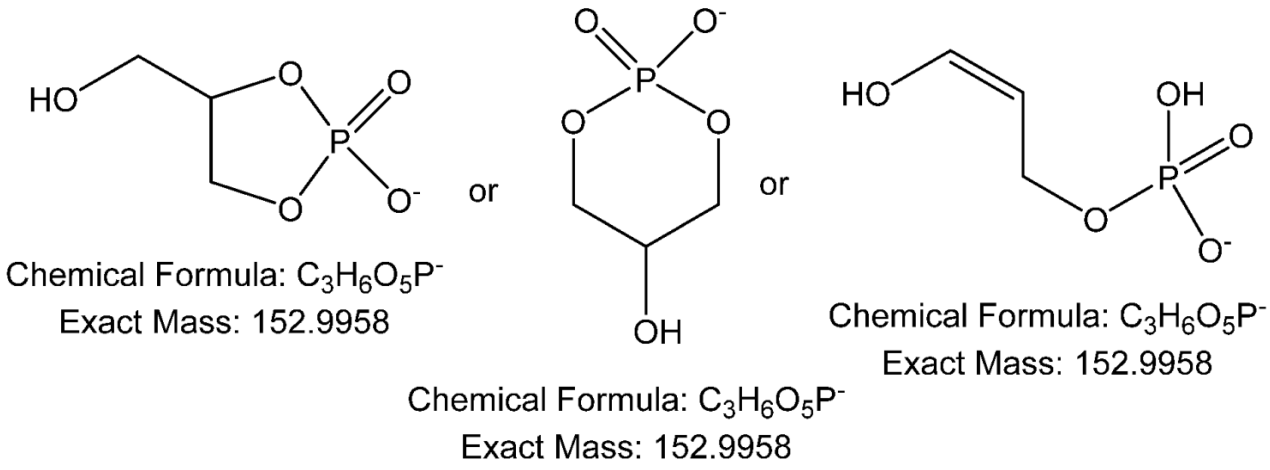

Figure 3S. The predicted structures of the ion at *m/z* 152.9958, used for ESI precursor ion scanning tandem mass spectrometry (PIS MS/MS).

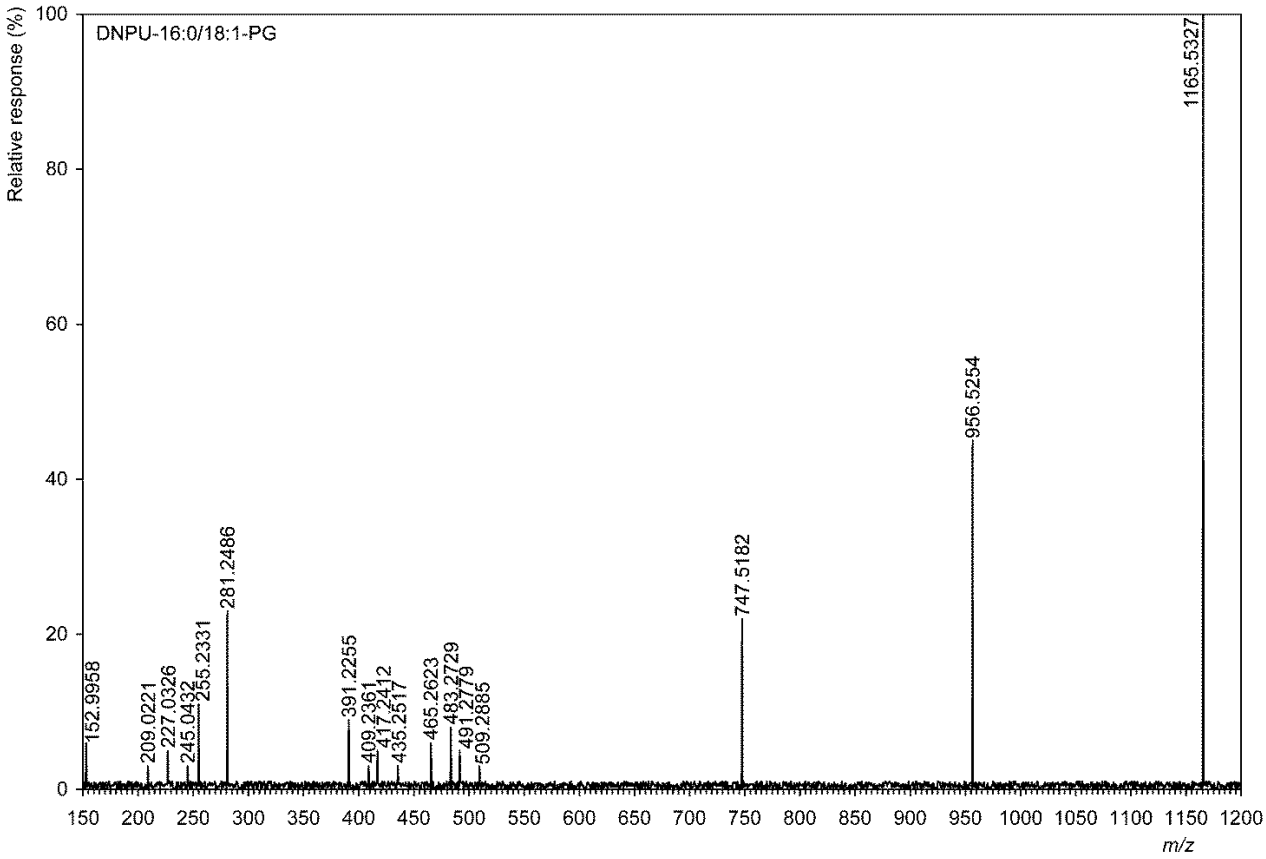

Figure 4S. High-resolution tandem mass spectrum of molecular species at *m/z* 1165.5327, i.e. $[M\text{-}DNPU]^-$ of 16:0/18:1-PG by negative ESI.

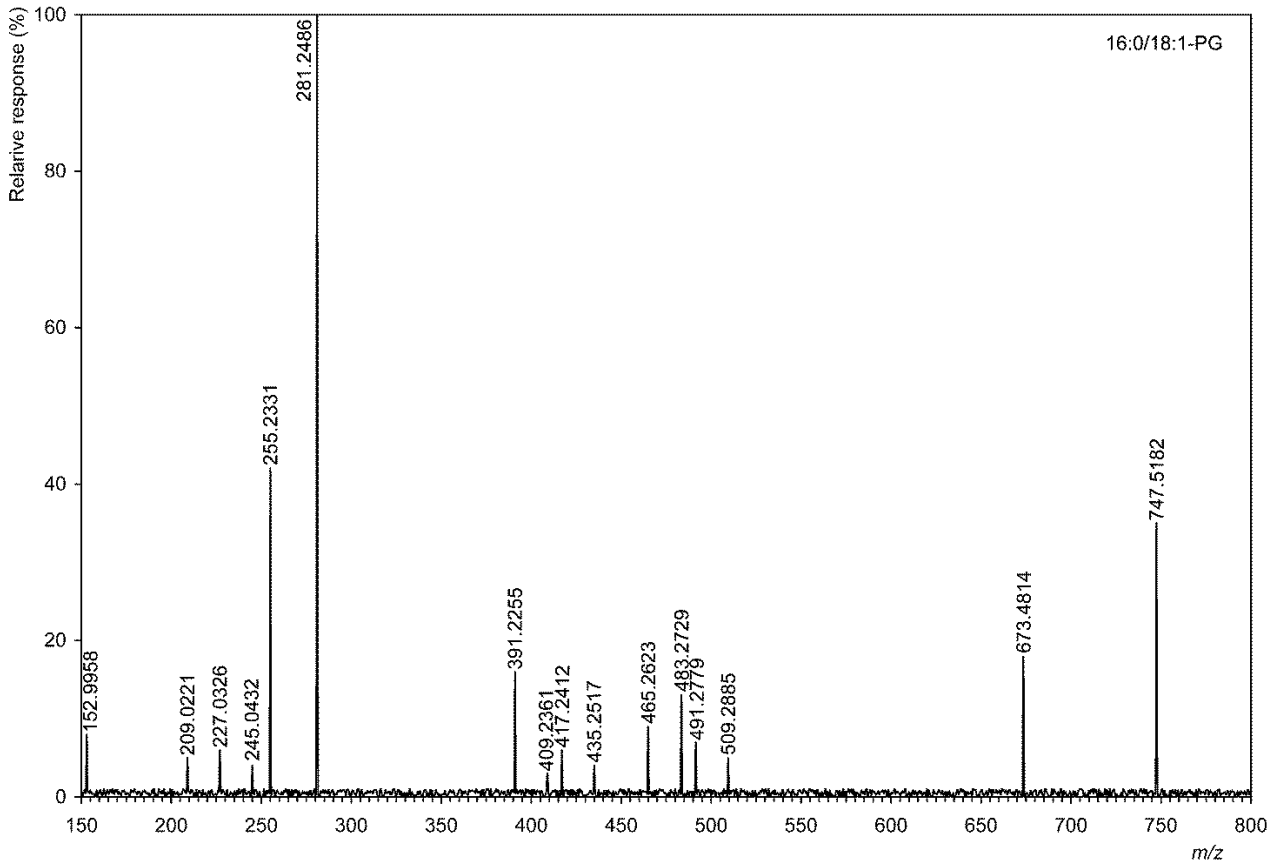

Figure 5S. High-resolution tandem mass spectrum of molecular species at *m/z* 747.5182, i.e. 16:0/18:1-PG, by negative ESI.

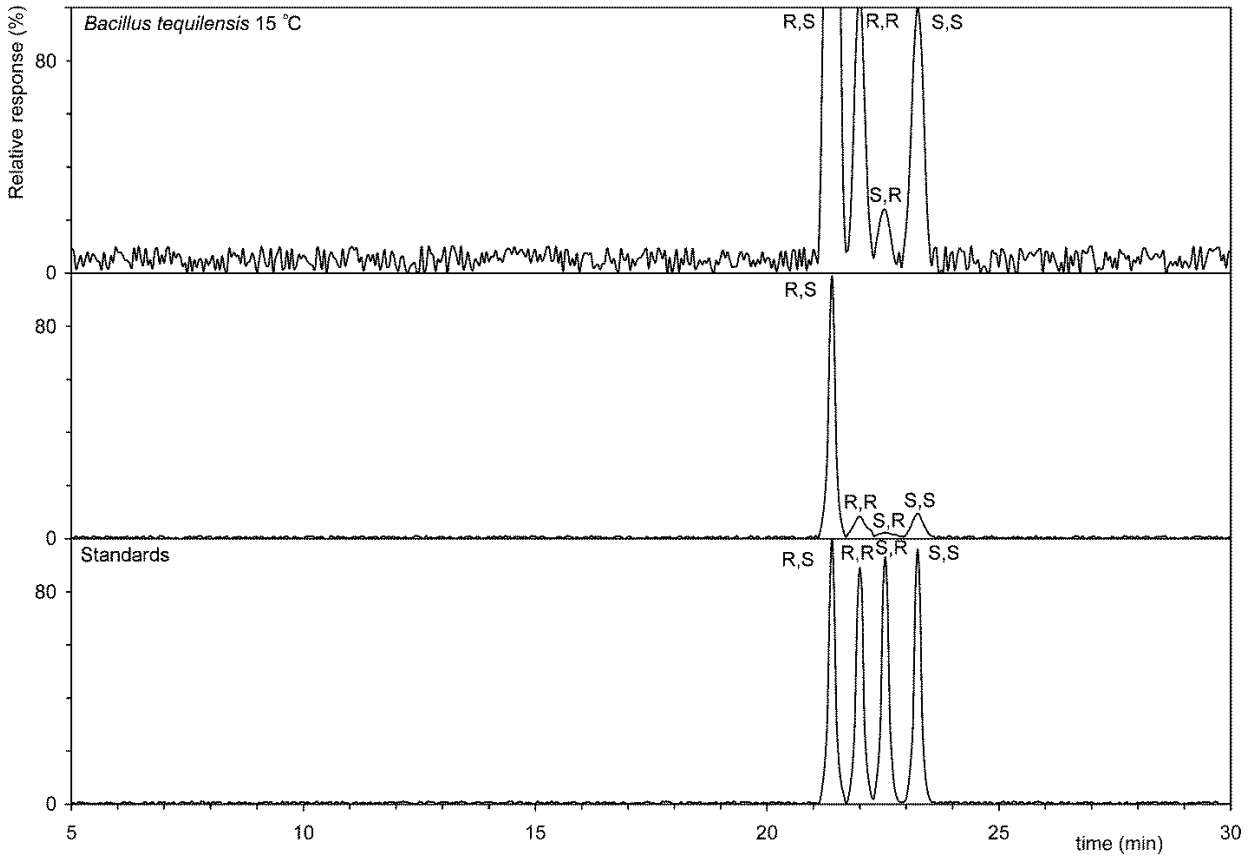

Figure 6S. Chiral-LC-MS of four PG isomers (*R,R, R,S, S,R*, and *S,S*) and bis(3,5-dinitrophenyl) urethanes of *Bacillus tequilensis*, cultivated at 15 °C. Intermediate chromatogram, relative response up to 100%, upper chromatogram, relative response, with increased sensitivity (10x).

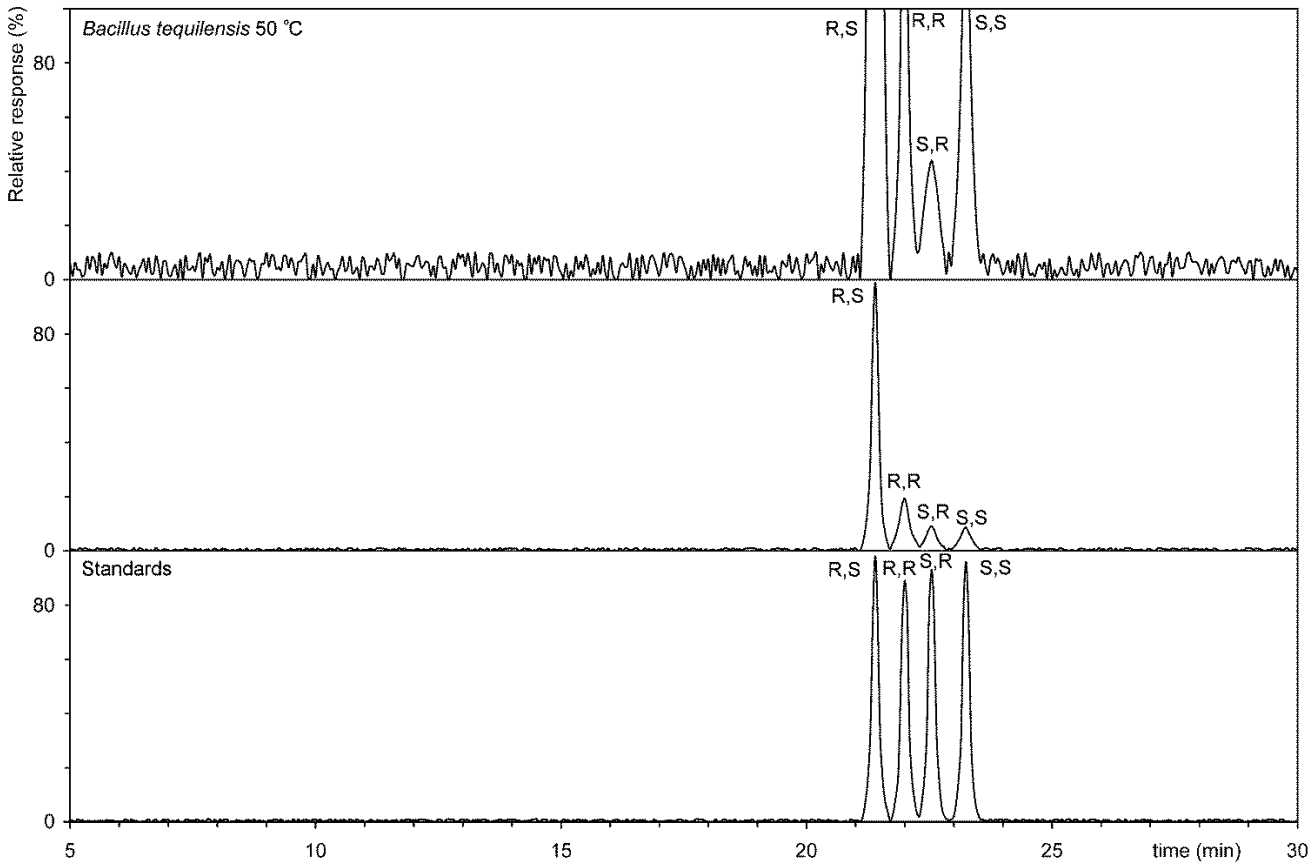

Figure 7S. Chiral-LC-MS of four PG isomers (*R,R, R,S, S,R*, and *S,S*) and bis(3,5-dinitrophenyl) urethanes from *Bacillus tequilensis*, cultivated at 50 °C. Intermediate chromatogram, relative response up to 100%, upper chromatogram, relative response, with increased sensitivity (10×).