Supporting Information for

PgpP is a broadly conserved phosphatase required for phosphatidylglycerol lipid synthesis

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Tables S1 to S6
Supporting text
SI References

Other supporting materials for this manuscript include the following:

Dataset S1
Dataset S2

Supporting Figures

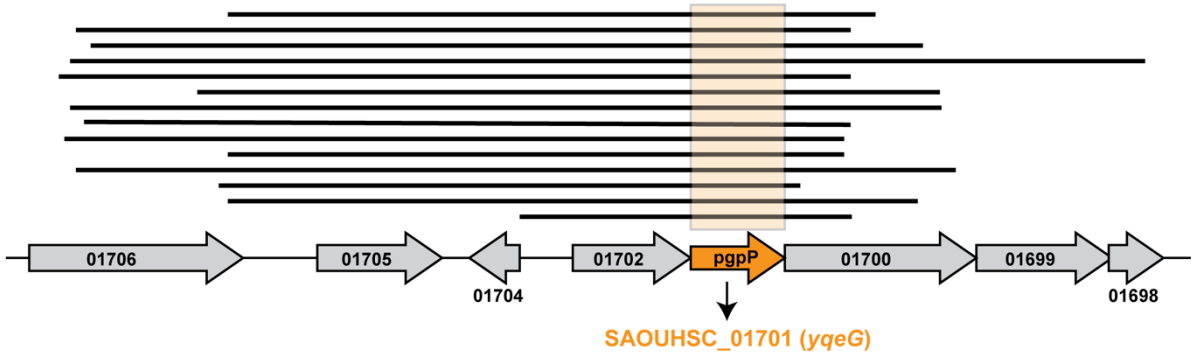


Figure S1. The *S. aureus* genomic regions that supported growth of an *E. coli pgp* mutant. As part of the 2022 and 2023 Cold Spring Harbor Advanced Bacterial Genetic course co-directed by the first author, the participating students selected for plasmids with *S. aureus* genes capable of suppressing the lethal phenotype of the *E. coli* Pgp-depletion strain grown under restrictive conditions. Black bars above the annotated region of the *S. aureus* genome represent the genomic fragments isolated from the genetic selection. The pUT18C-gDNA plasmids from *E. coli* YL24 pMAK-C clones that were able to grow at 43°C without arabinose were isolated, the inserts were sequenced and mapped onto the *S. aureus* NCTC8325 genome. Shown is the *S. aureus* genomic region with SAOUHSC locus tag numbers and some of the mapped reads. The gene with locus tag SAOUHSC_01701 (*yqeG*) and renamed *pgpP* is highlighted in orange and was identified as the best candidate gene coding for a Gram-positive PGP phosphatase.

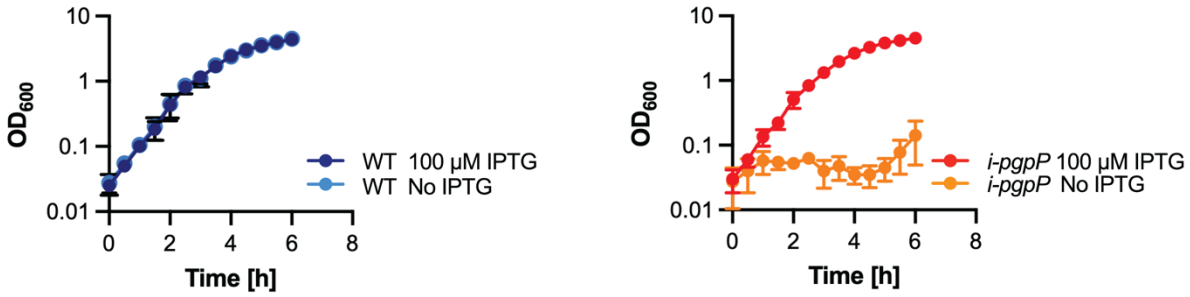


Figure S2. PgpP is essential for the growth of *B. subtilis.* Growth curves of the wild-type and inducible *pgpP* strains. Washed cultures of wild-type (WT) *B. subtilis* and the *i-pgpP* strain were used to inoculate LB medium without or with IPTG to an OD_{600} of 0.025 and the cultures were incubated for 3 h. At this point, the cultures were back diluted again to an OD_{600} of 0.025 into fresh LB medium without or with IPTG and the bacterial growth monitored by taking OD_{600} readings at timed intervals. The average reading and standard deviation from four independent cultures are plotted.

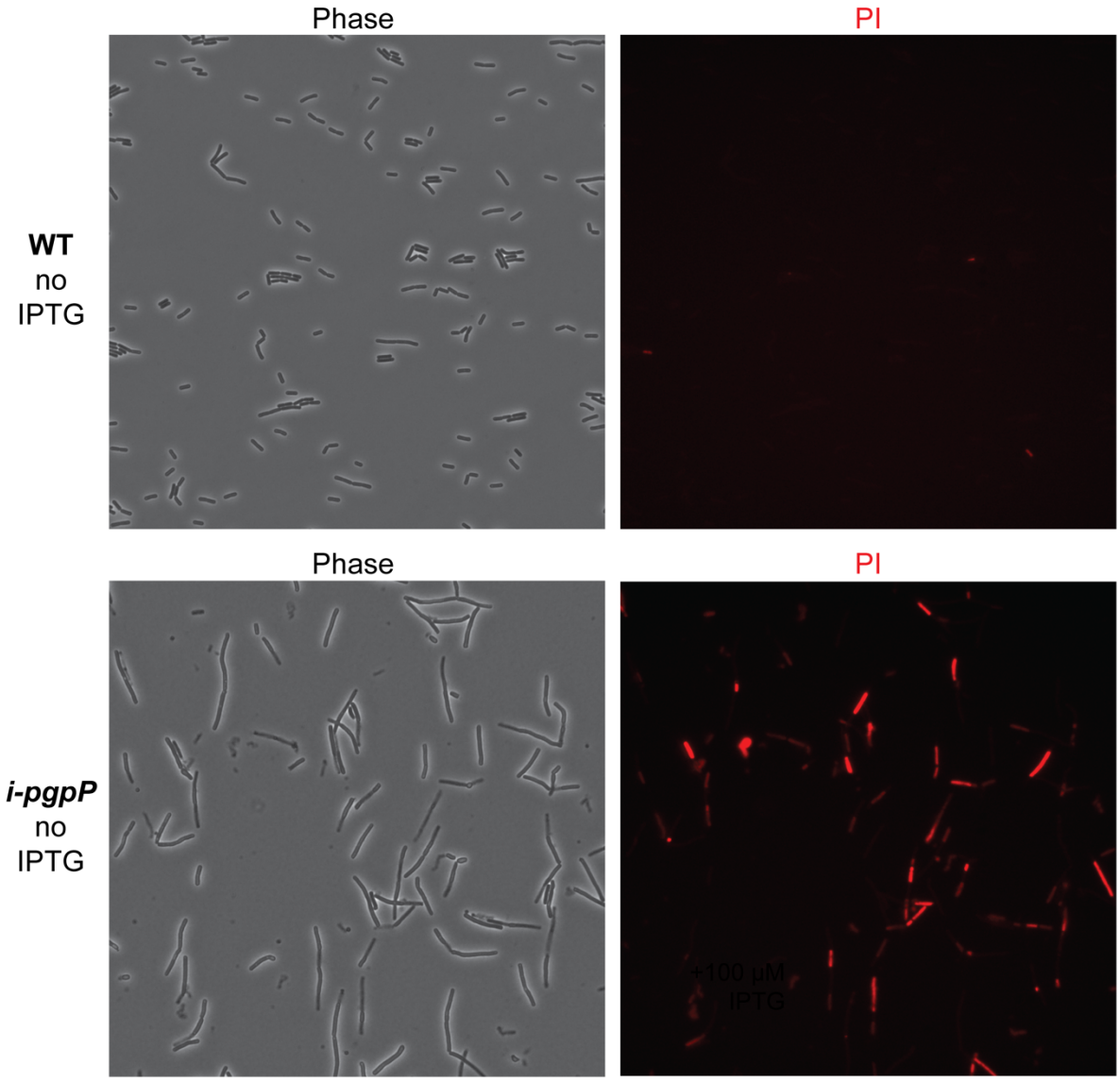


Figure S3: PgpP is essential in *B. subtilis* and its depletion leads to membrane defects. Representative phase-contrast and fluorescence images of wild-type (WT) *B. subtilis* and the *i-pgpP* strain. Cells grown for 5 h in the absence of IPTG were stained with propidium iodide (PI) and analyzed by phase-contrast and fluorescence microscopy. The exposure time and scaling for the two phase-contrast and two PI images are identical. Representative images from one of two independent experiments are shown.

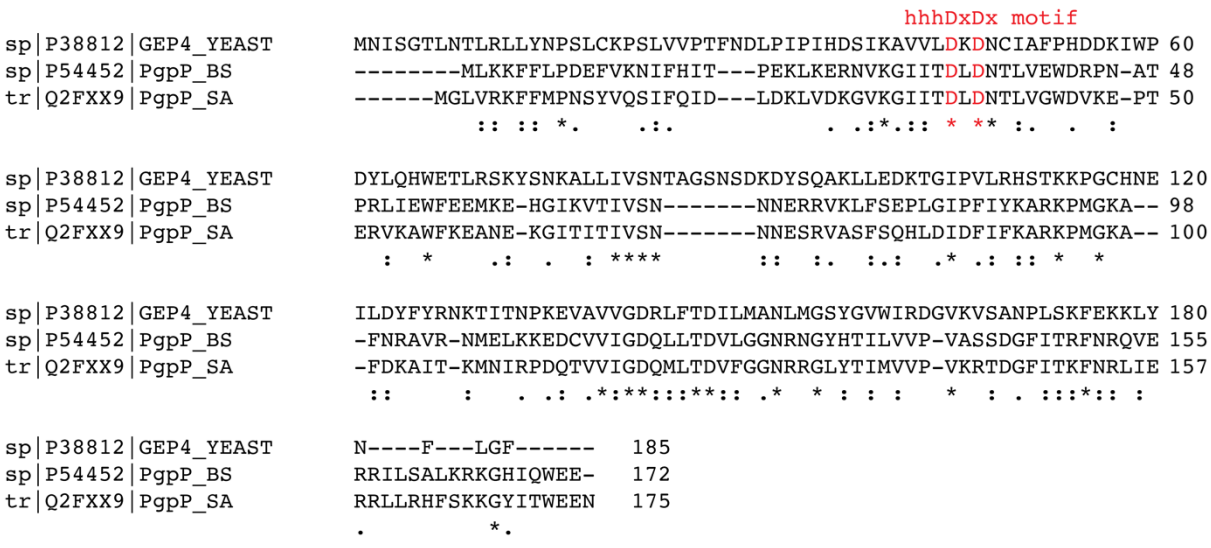


Figure S4. Sequence conservation between Gep4 and PgpP. The yeast mitochondrial PGP phosphatase Gep4 sequence (Uniprot P38812) was aligned with the *B. subtilis* PgpP (formerly, YqeG) (PgpP_BS; Unitprot P54452) and the *S. aureus* PgpP (PgpP_SA; Unitprot Q2FXX9) sequences using Clustal Omega (1). The two highly conserved aspartic acids within the active site motif hhhDxDx, where h indicates a hydrophobic amino acid and x any amino acid, are highlighted in red.

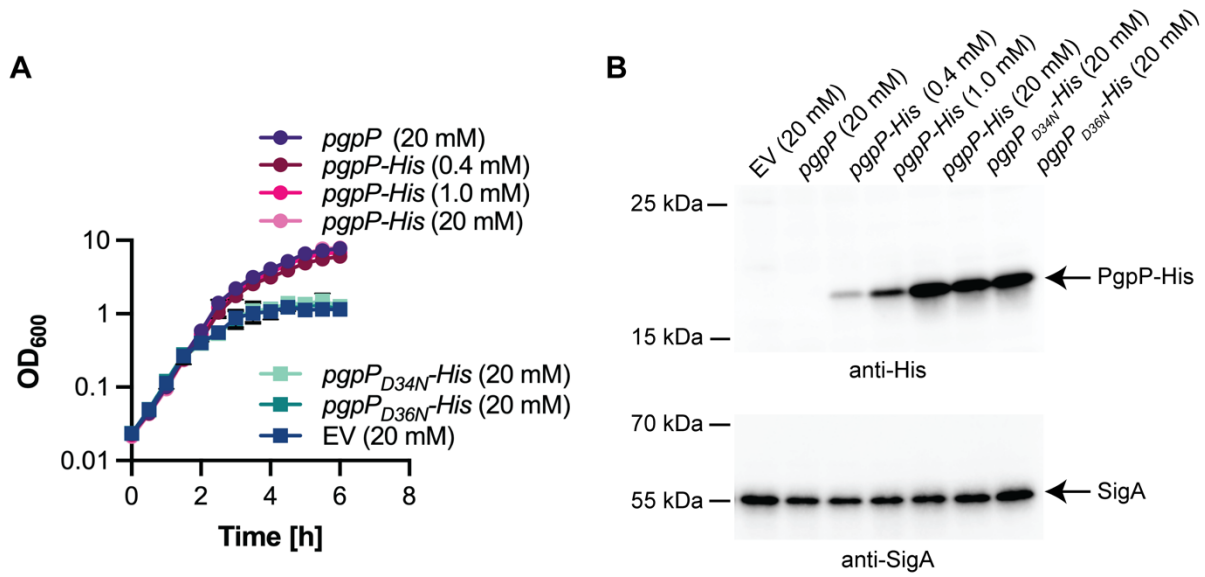


Figure S5. The PgpP$_{D34N}$ and PgpP$_{D36N}$ mutants are stable but nonfunctional. (A) Growth curves of the IPTG-regulated *pgpP* mutant (*i-pgpP*) containing an empty vector (EV) or the indicated xylose-regulated *pgpP, pgpP-His*, *pgpP$_{D34N}$-His* or *pgpP$_{D36N}$-His* variant. Cells were precultured in the presence of IPTG and then diluted into LB medium with the indicated concentrations of xylose and incubated with shaking at 37°C. OD_{600} readings were taken every 30 min for 6 h and average and standard deviation from three experiments plotted. Note that most error bars are too small to be visible on the graph. (B) Immunoblot analysis of PgpP-His variants. Samples were taken from the 3 h time point from cultures grown as shown in panel (A). C-terminally His tagged PgpP proteins were detected with a His-tag specific antibody and the SigA protein was detected as loading control. The final three lanes of the PgpP-His immunoblot are also shown in Fig. 4C. The experiment was performed in triplicate and representative immunoblots are shown.

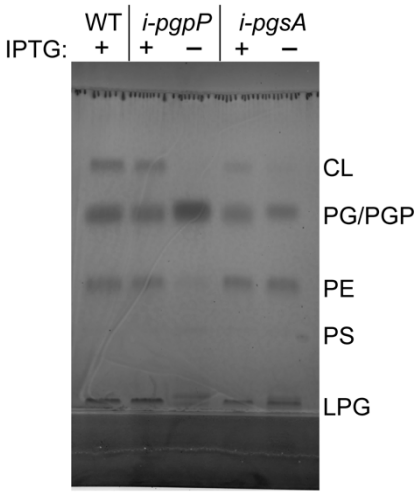


Figure S6: Depletion of PgpP leads to an altered membrane lipid profile. Thin layer chromatography (TLC) of total lipids extracted from WT *B. subtilis*, the PgpP depletion strain (*i-pgpP*) and PgsA depletion control strain (*i-pgsA*) following growth in LB medium with or without IPTG as indicated. The lipids were separated by TLC using a chloroform:methanol:acetic acid solvent system and stained with primulin and the plate was subsequently imaged. The lipid bands were annotated as previously described by Roney and Rudner (2). PG and PGP appear to have very similar mobilities under these conditions and cannot be resolved. Similar as in the *i-pgsA* control strain, depletion of PgpP leads to a reduction in cardiolipin (CL) production. This is consistent with a defect in PG synthesis, since PG is the precursor for CL synthesis. In the PgpP depletion strain lower levels of phosphatidylethanolamine (PE) and an additional lipid with a slightly increased mobility compared to lysylphosphatidylglycerol (LPG) were observed. A representative image from three independent experiments is shown.

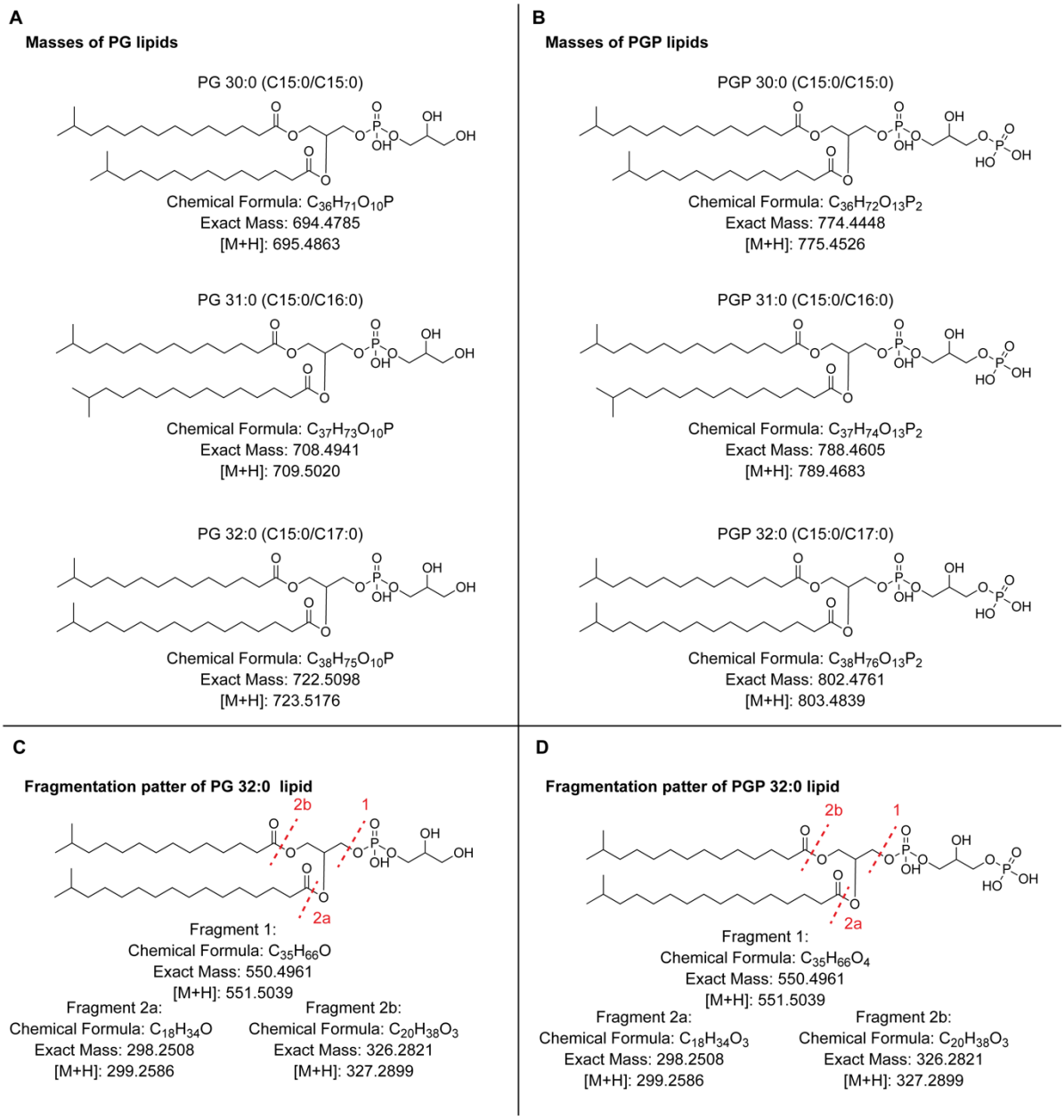


Figure S7. Chemical structures and MS/MS fragmentation pattern of PG and PGP lipids identified in *B. subtilis* membranes. (A-B) Chemical structures of *B. subtilis* (A) PG lipids with a combined fatty acid carbon chain length of 30:0; 31:0 or 32:0 and (B) PGP lipids with a combined fatty acid carbon chain length of 30:0, 31:0 or PGP 32:0 with chemical formulas, exact masses and M+H shown. (C-D) Schematic of the MS/MS fragmentation pattern of (C) PG 32:0 and (D) PGP 32:0 lipid with chemical formulas, exact masses and M+H for the different fragments shown. Fragment 1 is derived following the loss of the head group; Fragments 2a and 2b are obtained following the loss of the head group and one or the other fatty acid.

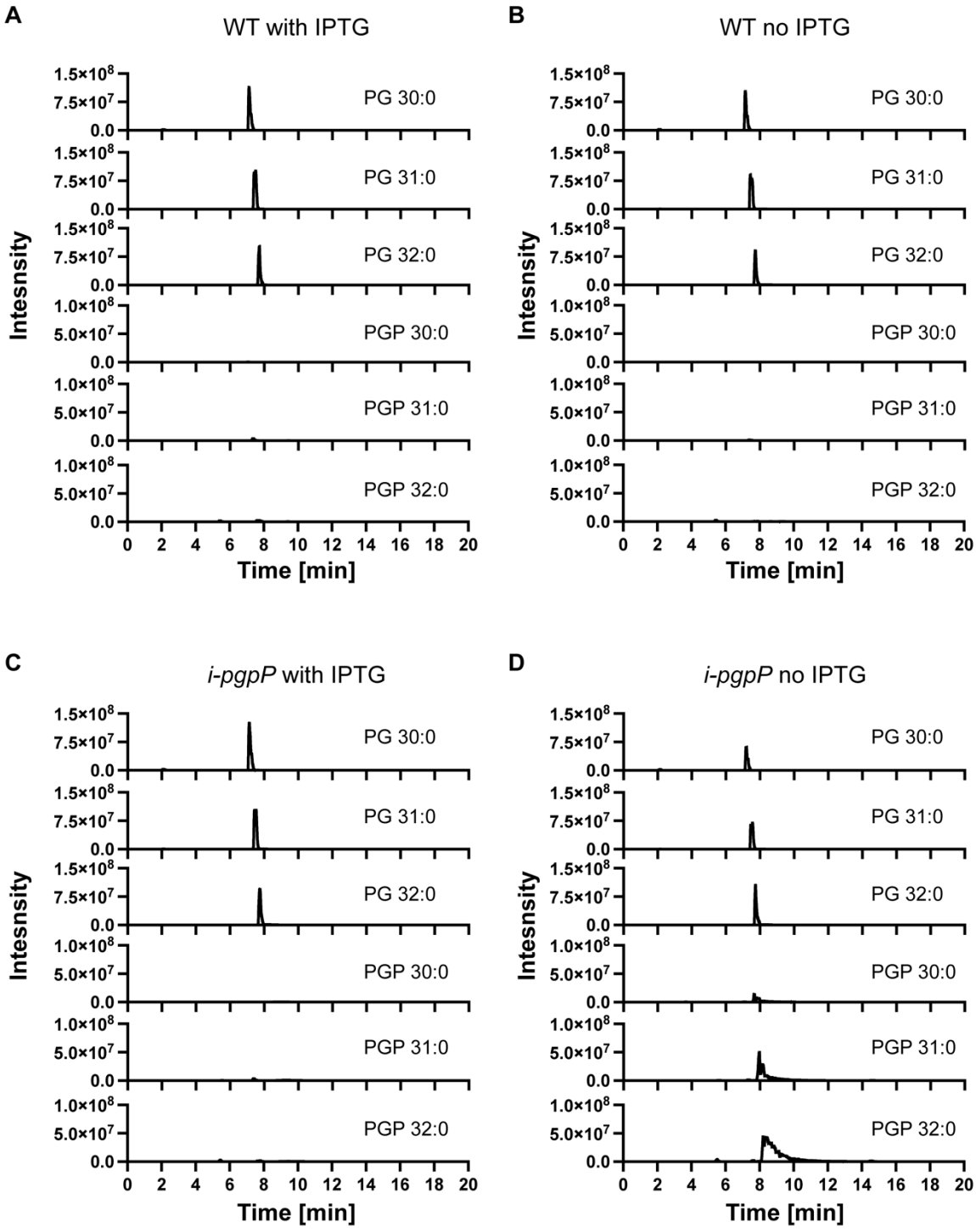


Figure S8. Extracted mass traces for PG and PGP lipids. Total membrane lipids were isolated from (A) WT PY79 grown in LB with IPTG, (B) WT PY79 grown in LB no IPTG, (C) PY79 *i-pgpP* grown in LB with IPTG and (D) PY79 *i-pgpP* grown in LB no IPTG and analyzed by mass spectrometry in the positive ion mode. Extracted mass traces for PG or PGP lipids with a combined fatty acid carbon chain length of 30:0, 31:0 or 32:0 are shown. One representative result from three experiments is shown and cropped versions of the WT no IPTG (B) and *i-pgpP* no IPTG (D) traces are also shown in Fig. 5C.

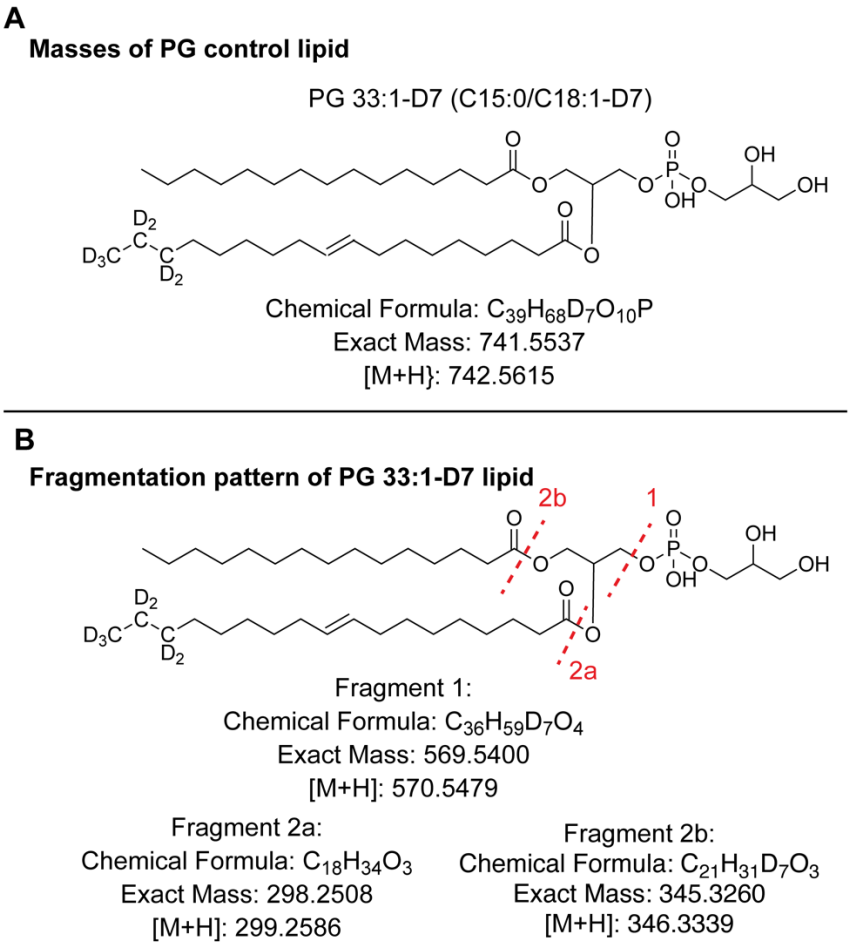


Figure S9. Chemical structure and MS/MS fragmentation pattern of the PG 33:1-D7 control lipid. (A) The chemical structure of the PG 33:1-D7 control lipid with C15:0 and C18:1-D7 fatty acid chains is shown along with the chemical formula, exact mass and M+H. (B) Schematic of the MS/MS fragmentation pattern of the PG 33:1-D7 lipid is shown with chemical formula, exact mass and M+H for the different fragments indicated. Fragment 1 is derived following the loss of the head group; Fragments 2a and 2b are obtained following the loss of the head group and one or the other fatty acid.

PG 33:1-D7 (C15:0 C18:1-D7) MS/MS

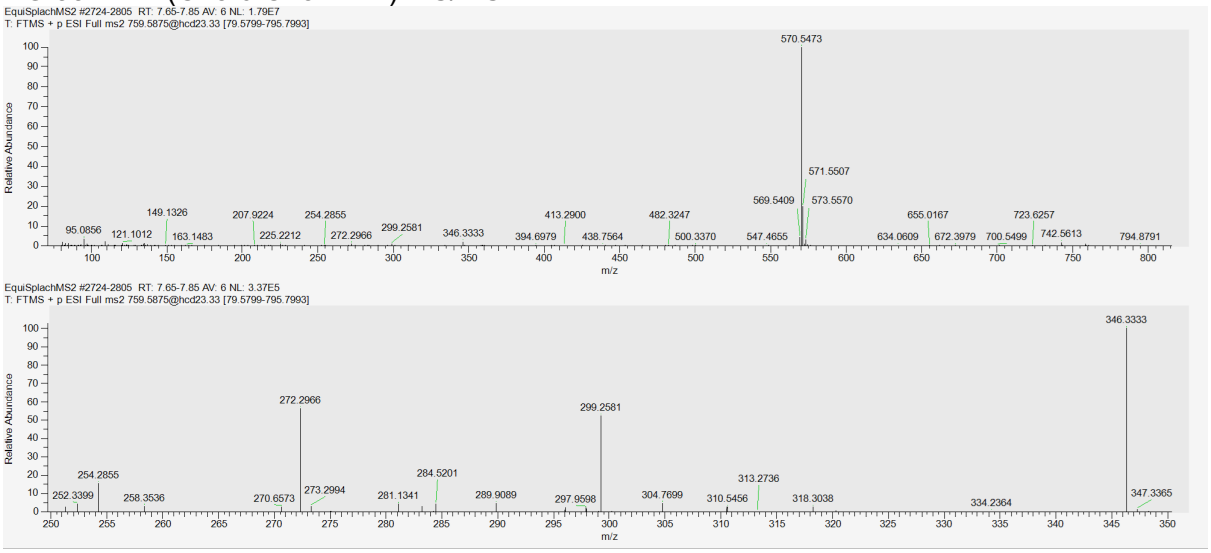
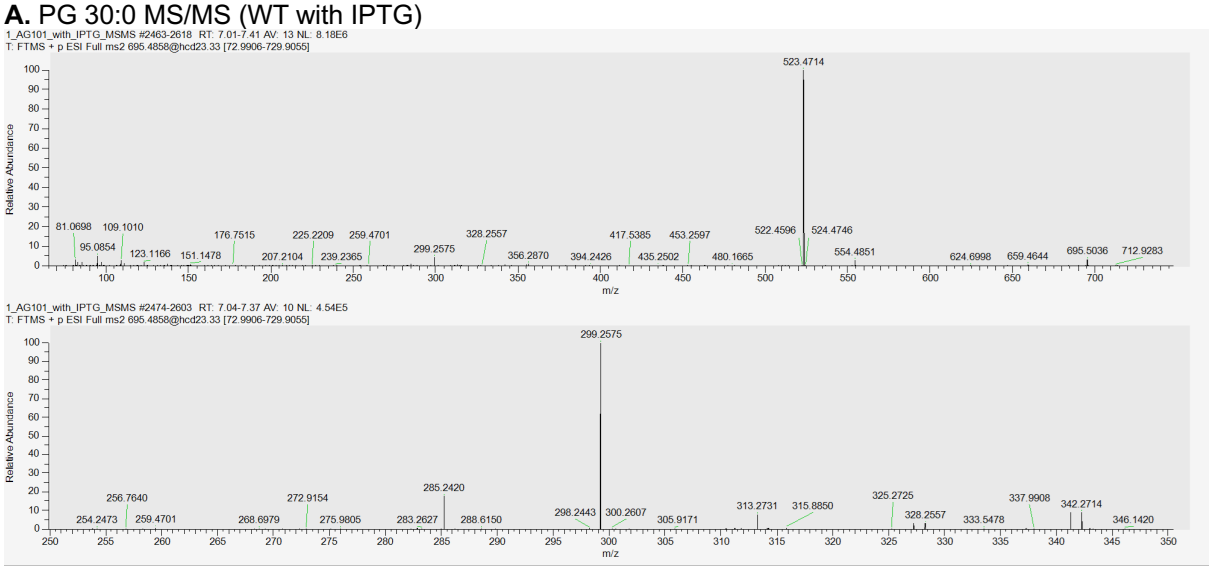
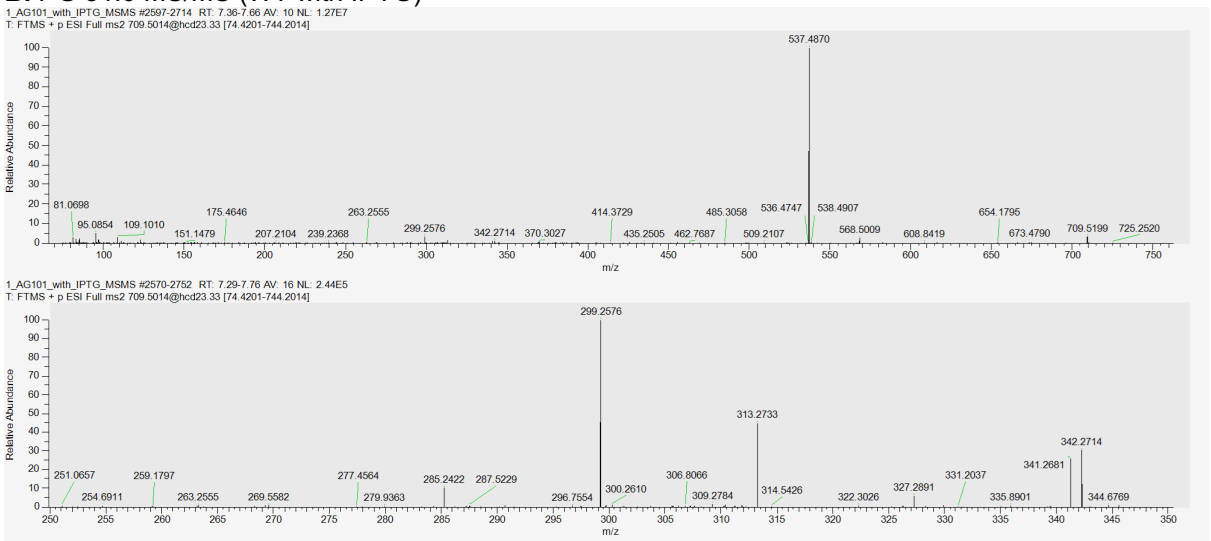


Figure S10. MS/MS spectra for the PG 33:1-D7 (C15:0 C18:1-D7) control lipid. Top panel is the full range spectrum showing the mass signal for Fragment 1 (loss of head group) and the bottom panel is a zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

A. PG 30:0 MS/MS (WT with IPTG)



B. PG 31:0 MS/MS (WT with IPTG)



C. PG 32:0 MS/MS (WT with IPTG)

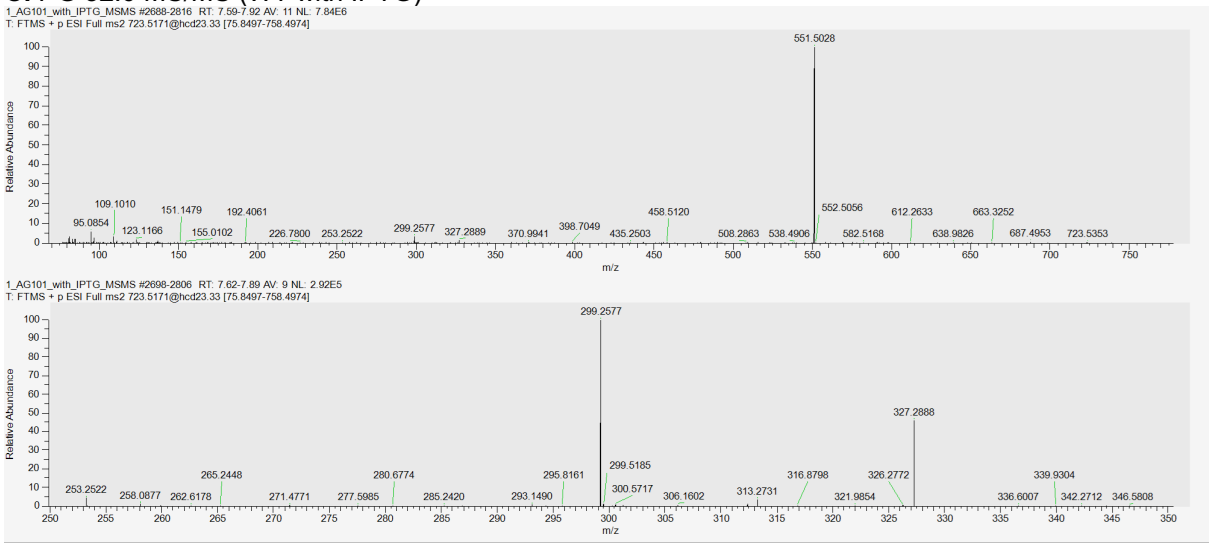
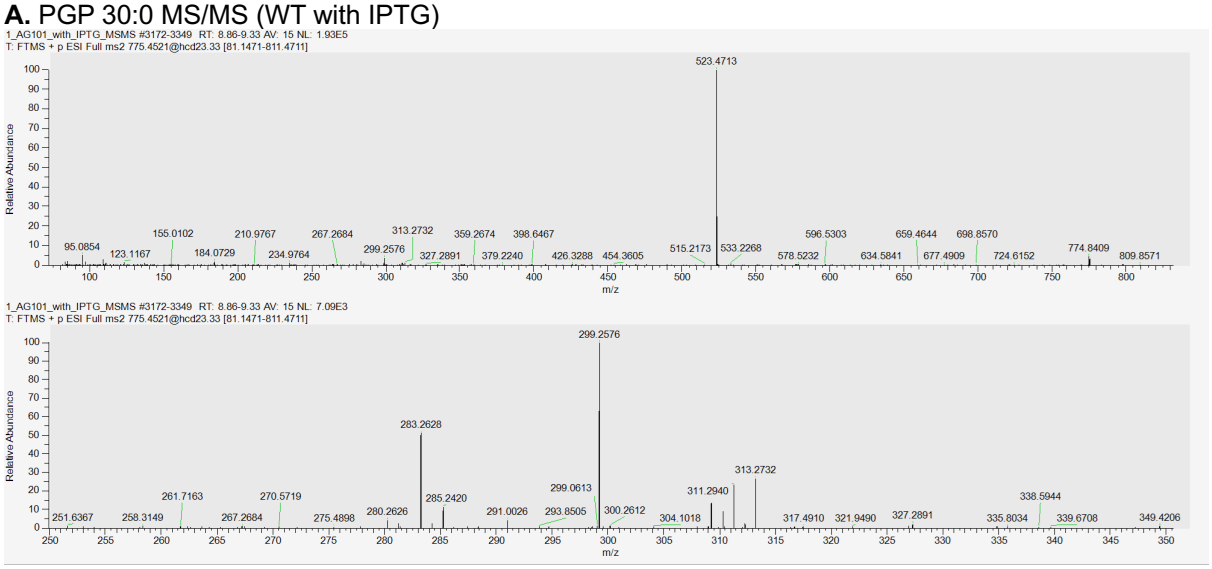
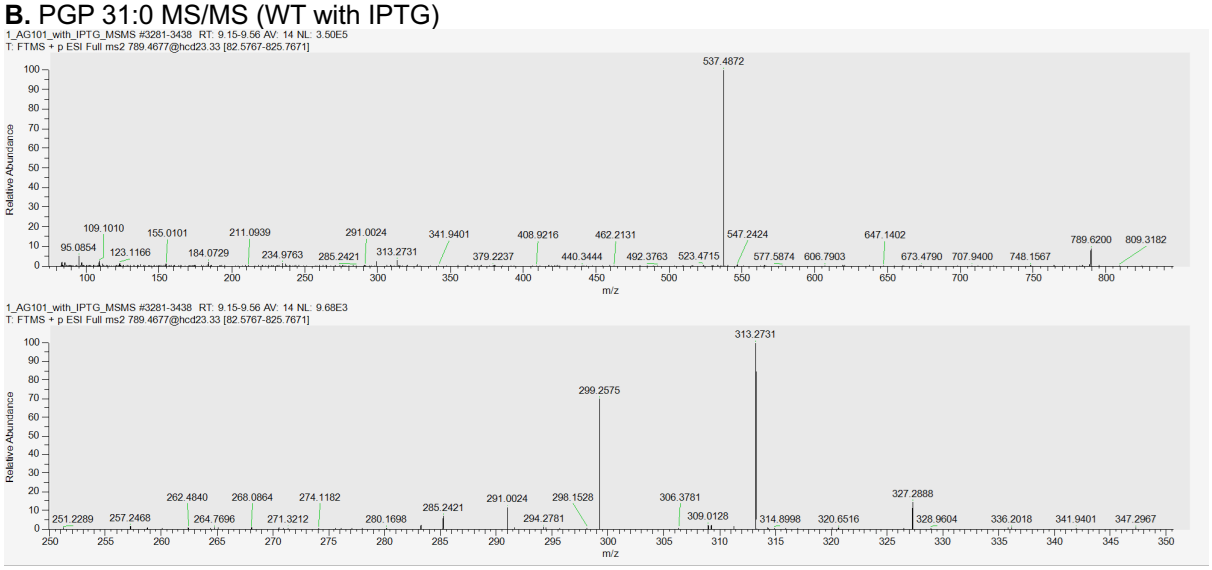


Figure S11. MS/MS spectra for (A) PG 30:0, (B) PG 30:1, (C) PG 32:0 lipids isolated from WT PY79 grown in the presence of IPTG. Top panels are full range spectra showing the mass signal for Fragment 1 (loss of head group) and bottom panels are zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

A. PGP 30:0 MS/MS (WT with IPTG)



B. PGP 31:0 MS/MS (WT with IPTG)



C. PGP 32:0 MS/MS (WT with IPTG)

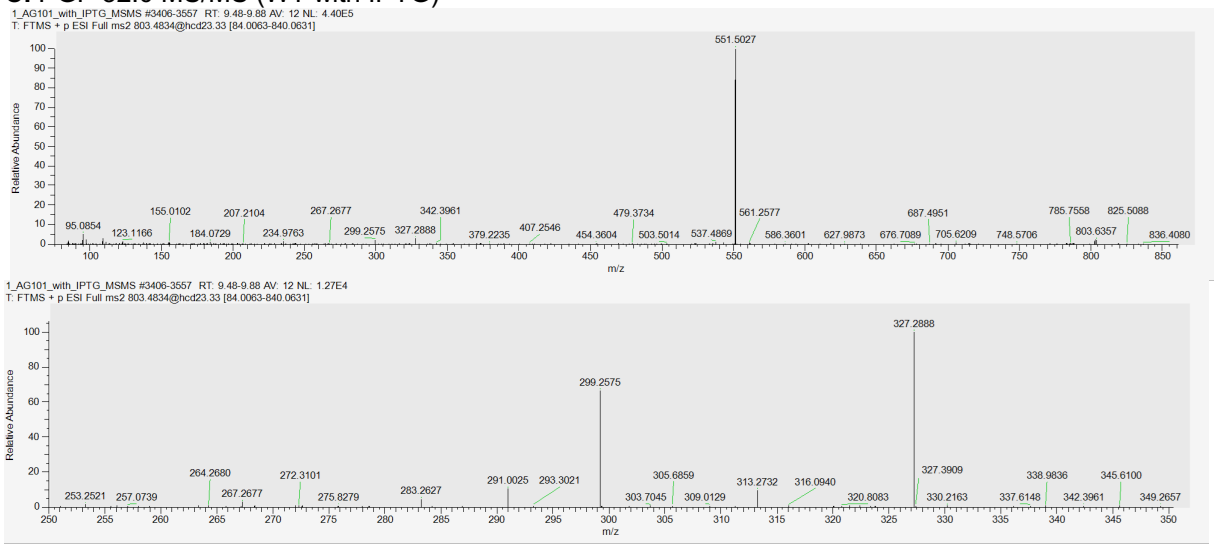
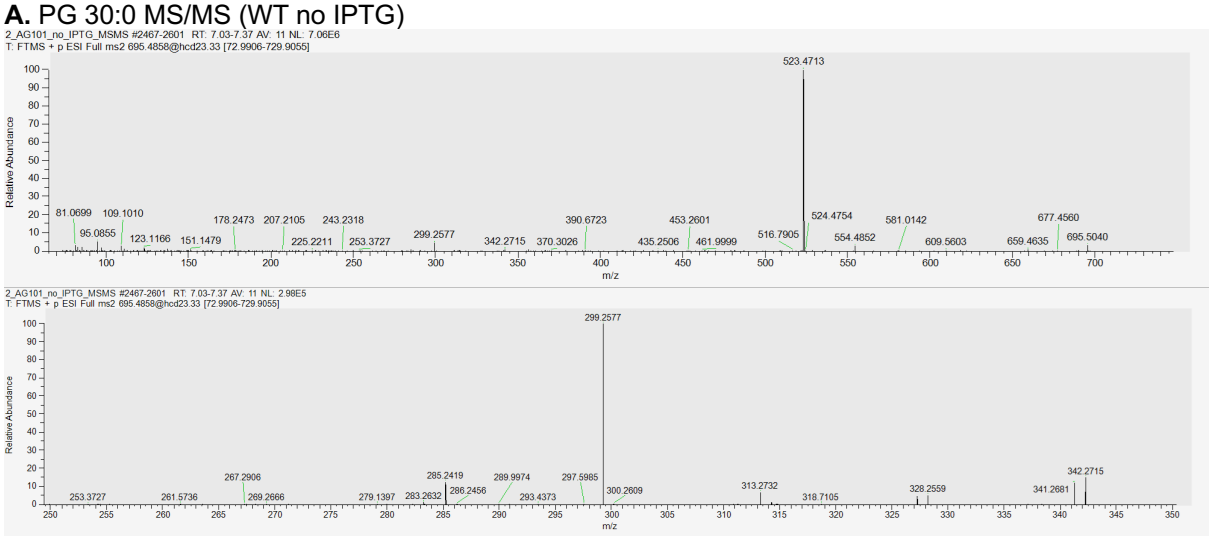
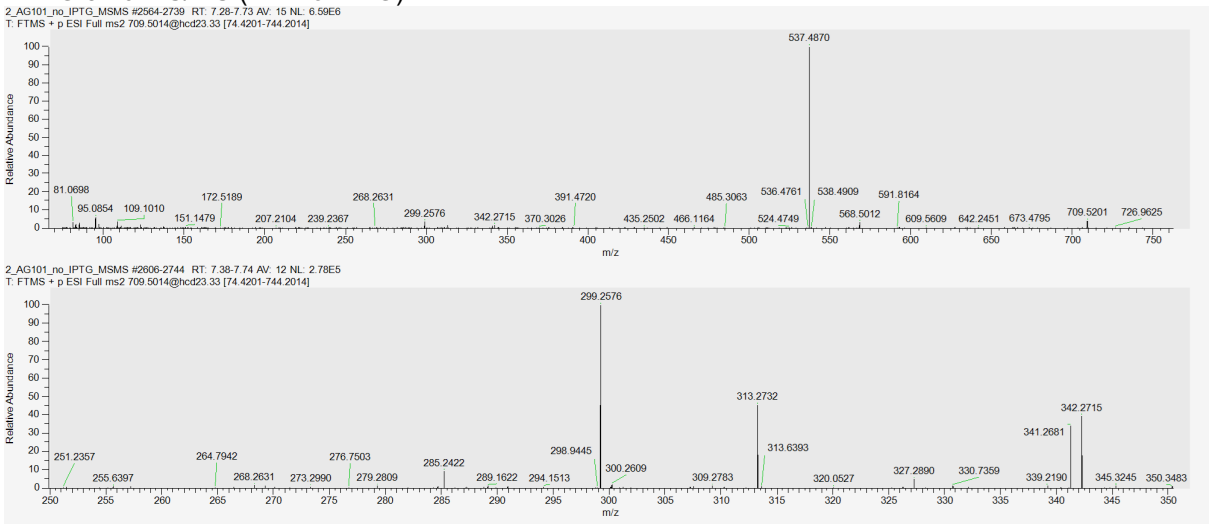


Figure S12. MS/MS spectra for (A) PGP 30:0, (B) PGP 30:1, (C) PGP 32:0 lipids isolated from WT PY79 grown in the presence of IPTG. Top panels are full range spectra showing the mass signal for Fragment 1 (loss of head group) and bottom panels are zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

A. PG 30:0 MS/MS (WT no IPTG)



B. PG 31:0 MS/MS (WT no IPTG)



C. PG 32:0 MS/MS (WT no IPTG)

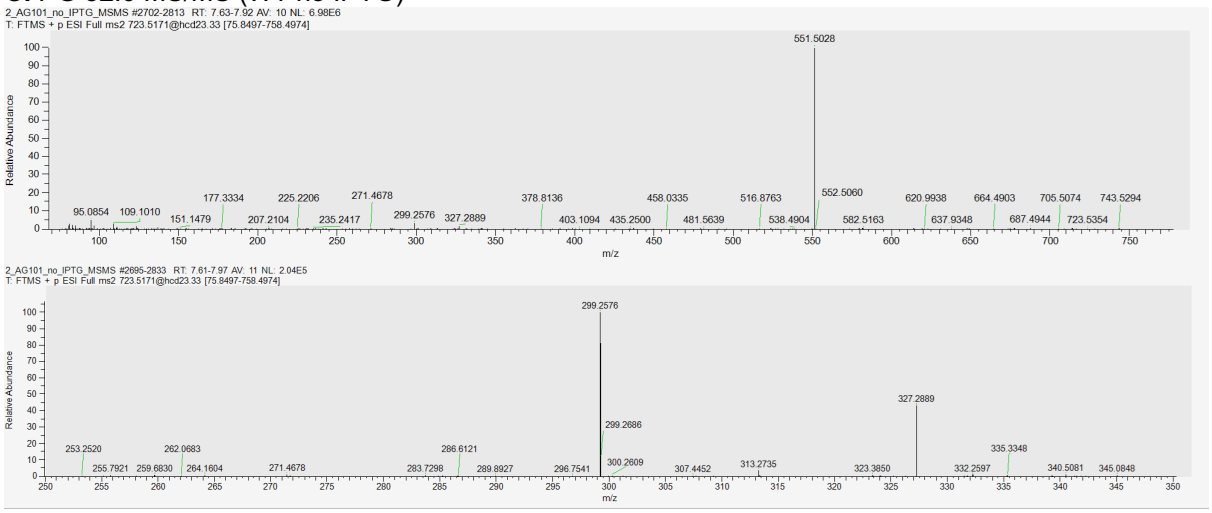
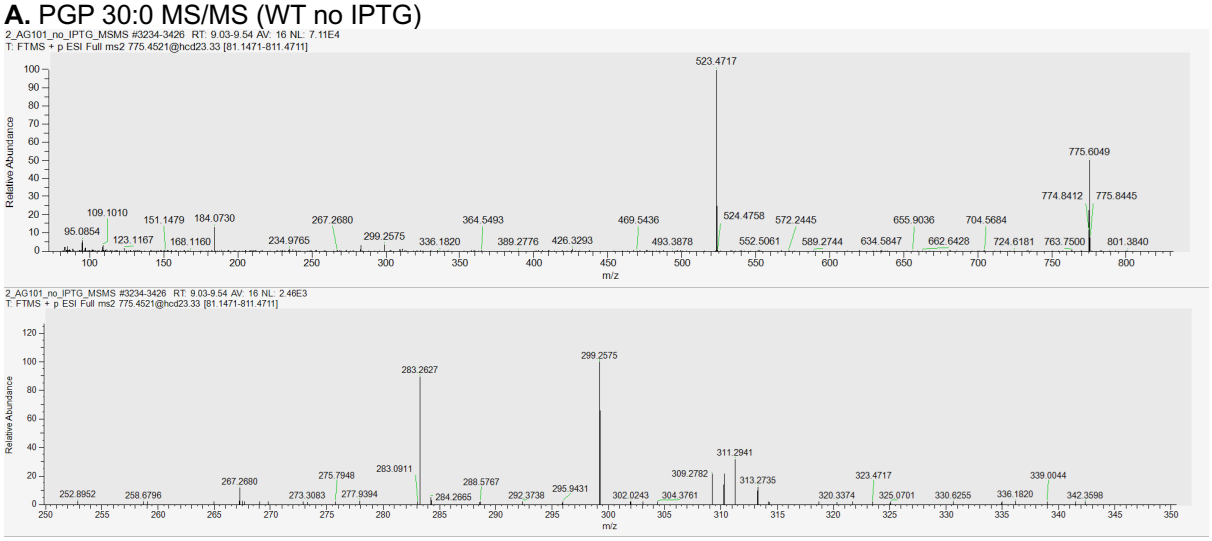
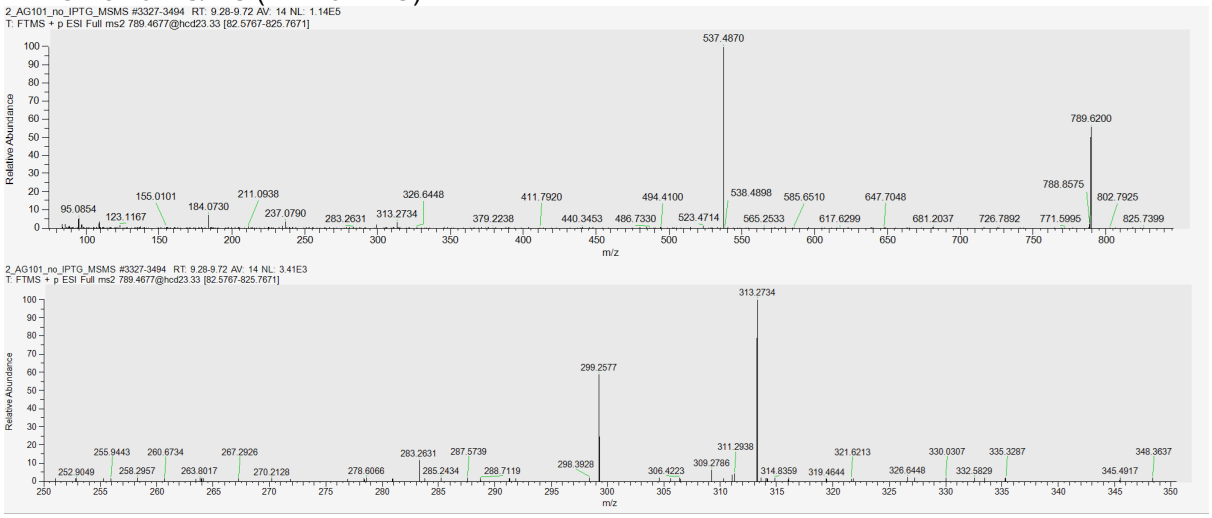


Figure S13. MS/MS spectra for (A) PG 30:0, (B) PG 30:1, (C) PG 32:0 lipids isolated from WT PY79 grown in the absence of IPTG. Top panels are full range spectra showing the mass signal for Fragment 1 (loss of head group) and bottom panels are zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

A. PGP 30:0 MS/MS (WT no IPTG)



B. PGP 31:0 MS/MS (WT no IPTG)



C. PGP 32:0 MS/MS (WT no IPTG)

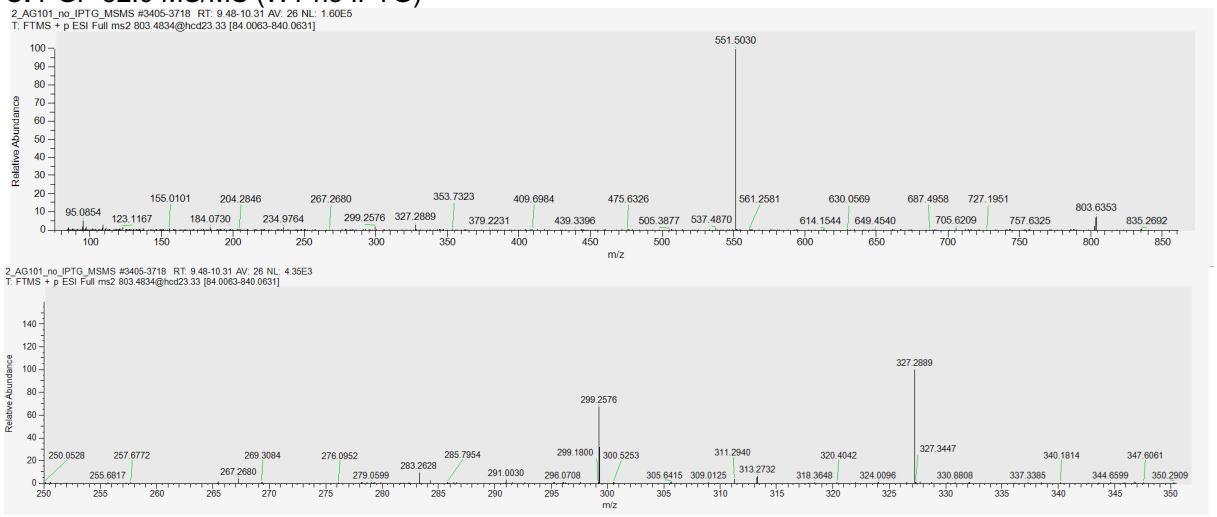
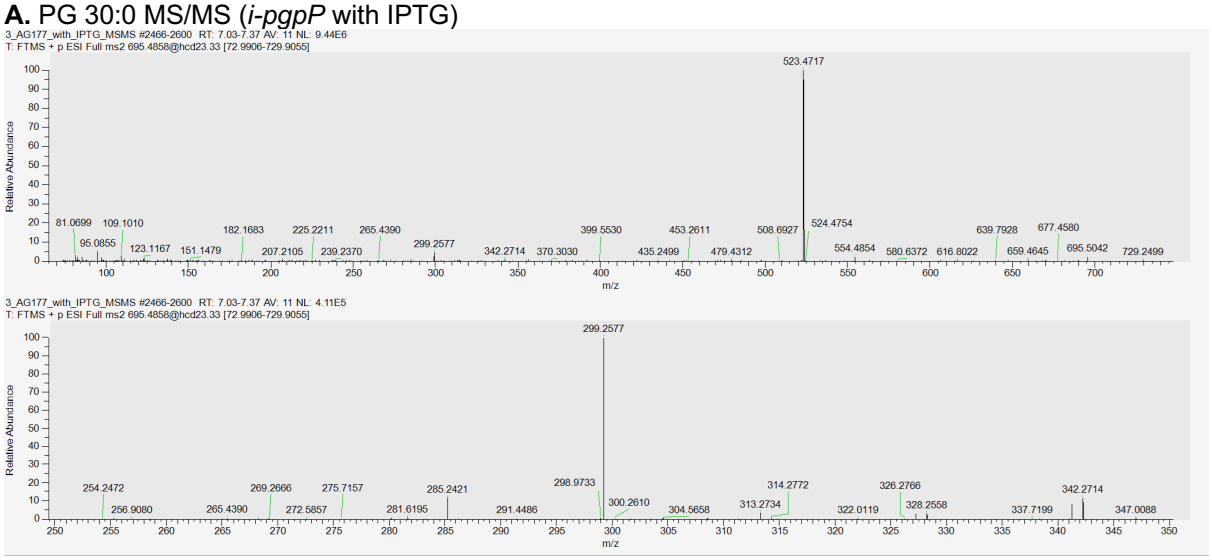
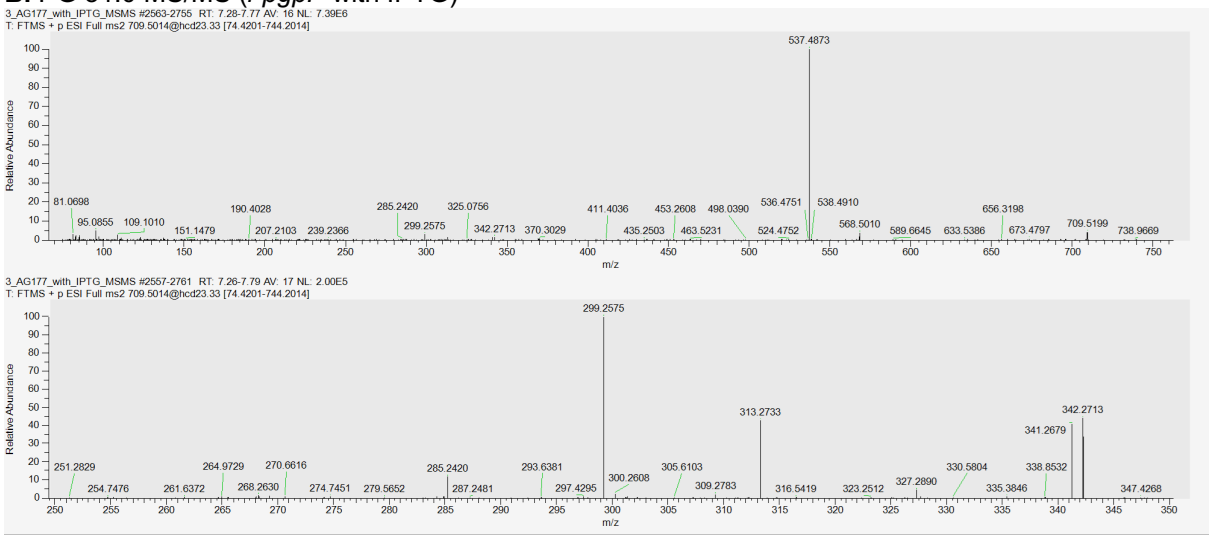


Figure S14. MS/MS spectra for (A) PGP 30:0, (B) PGP 30:1, (C) PGP 32:0 lipids isolated from WT PY79 grown in the absence of IPTG. Top panels are full range spectra showing the mass signal for Fragment 1 (loss of head group) and bottom panels are zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

A. PG 30:0 MS/MS (*i-pgpP* with IPTG)



B. PG 31:0 MS/MS (*i-pgpP* with IPTG)



C. PG 32:0 MS/MS (*i-pgpP* with IPTG)

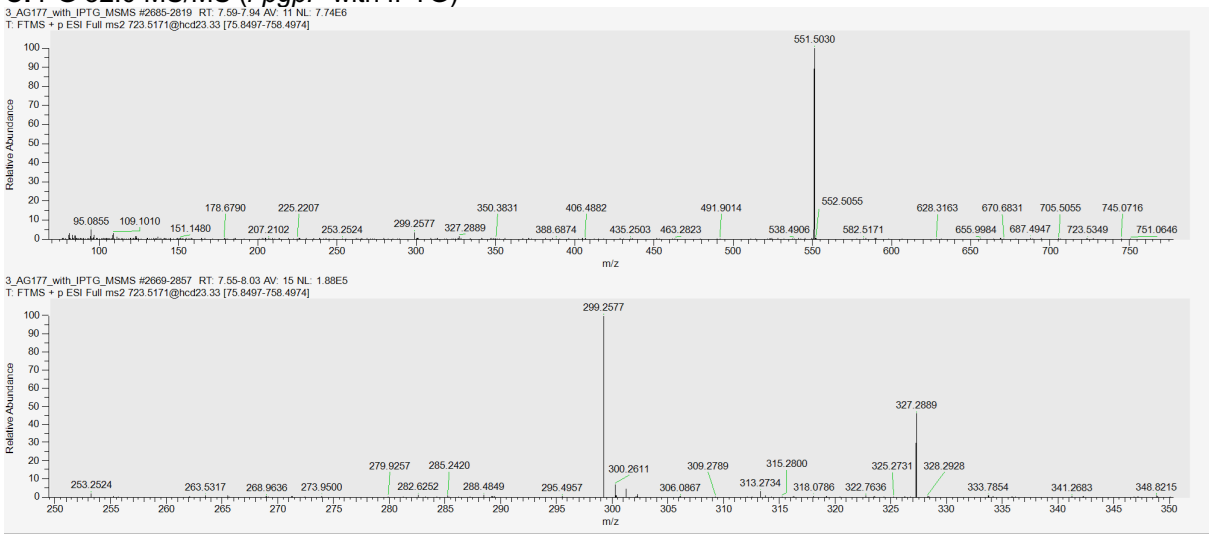
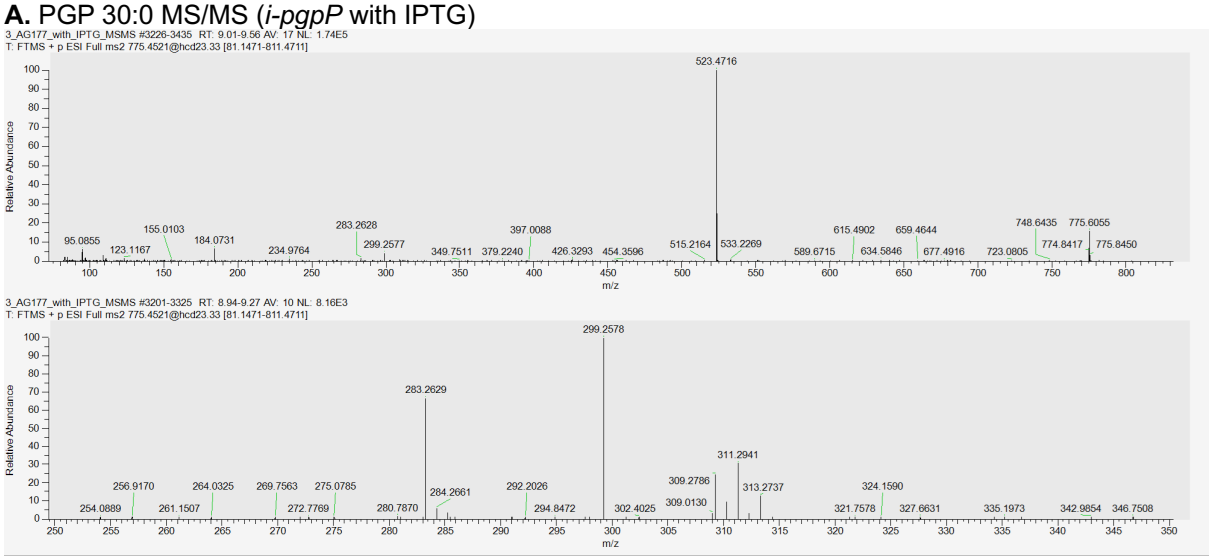
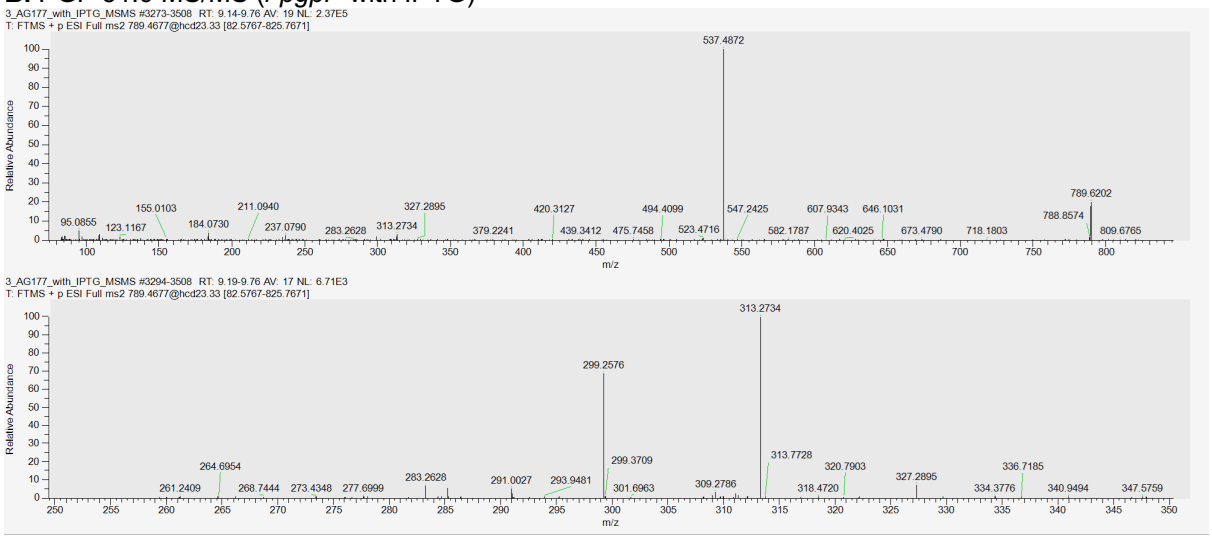


Figure S15. MS/MS spectra for (A) PG 30:0, (B) PG 30:1, (C) PG 32:0 lipids isolated from strain PY79 *i-pgpP* grown in the presence of IPTG. Top panels are full range spectra showing the mass signal for Fragment 1 (loss of head group) and bottom panels are zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

A. PGP 30:0 MS/MS (*i-pgpP* with IPTG)



B. PGP 31:0 MS/MS (*i-pgpP* with IPTG)



C. PGP 32:0 MS/MS (*i-pgpP* with IPTG)

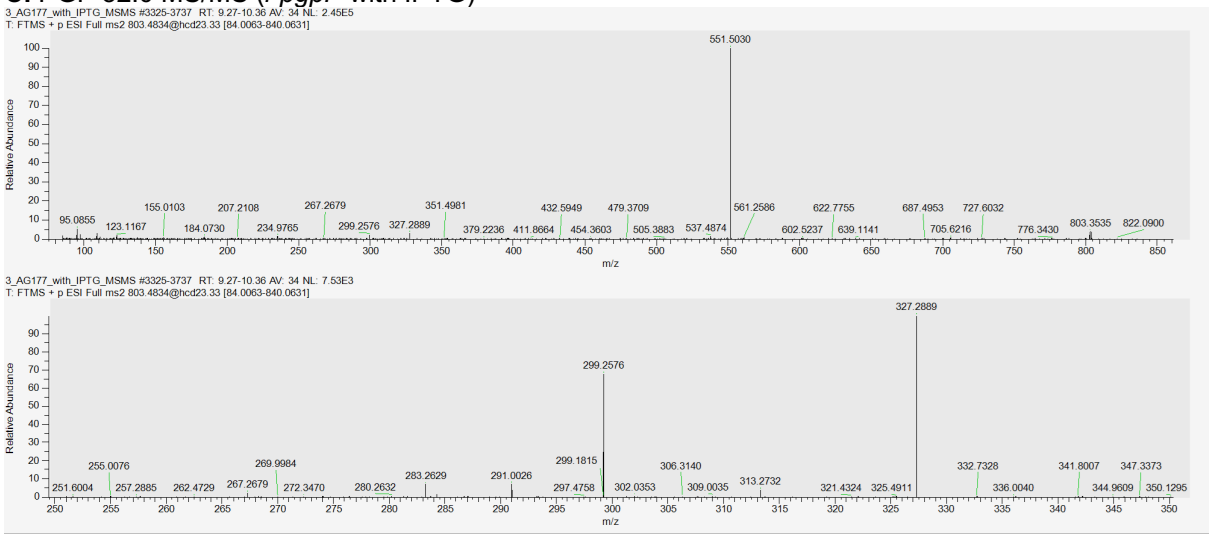
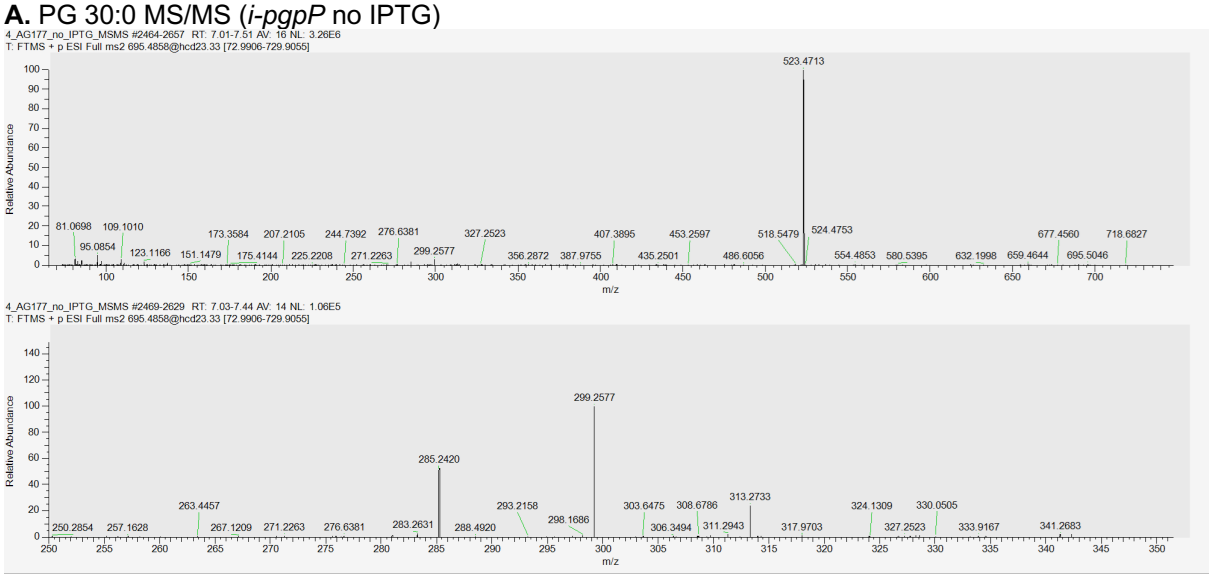
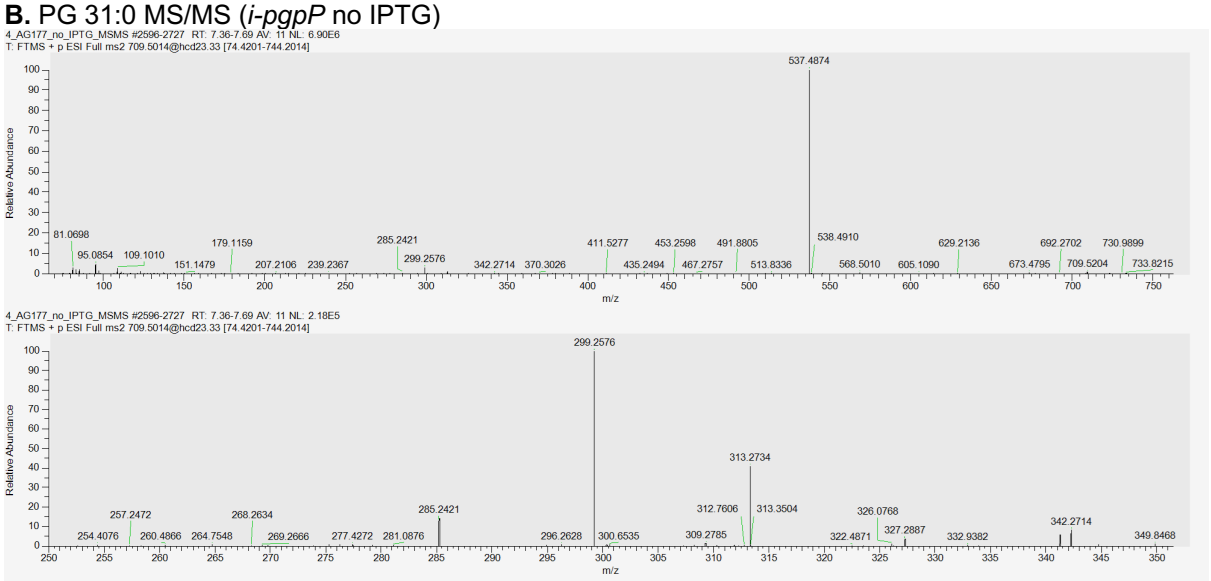


Figure S16. MS/MS spectra for (A) PGP 30:0, (B) PGP 30:1, (C) PGP 32:0 lipids isolated from strain PY79 *i-pgpP* grown in the presence of IPTG. Top panels are full range spectra showing the mass signal for Fragment 1 (loss of head group) and bottom panels are zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

A. PG 30:0 MS/MS (*i-pgpP* no IPTG)



B. PG 31:0 MS/MS (*i-pgpP* no IPTG)



C. PG 32:0 MS/MS (*i-pgpP* no IPTG)

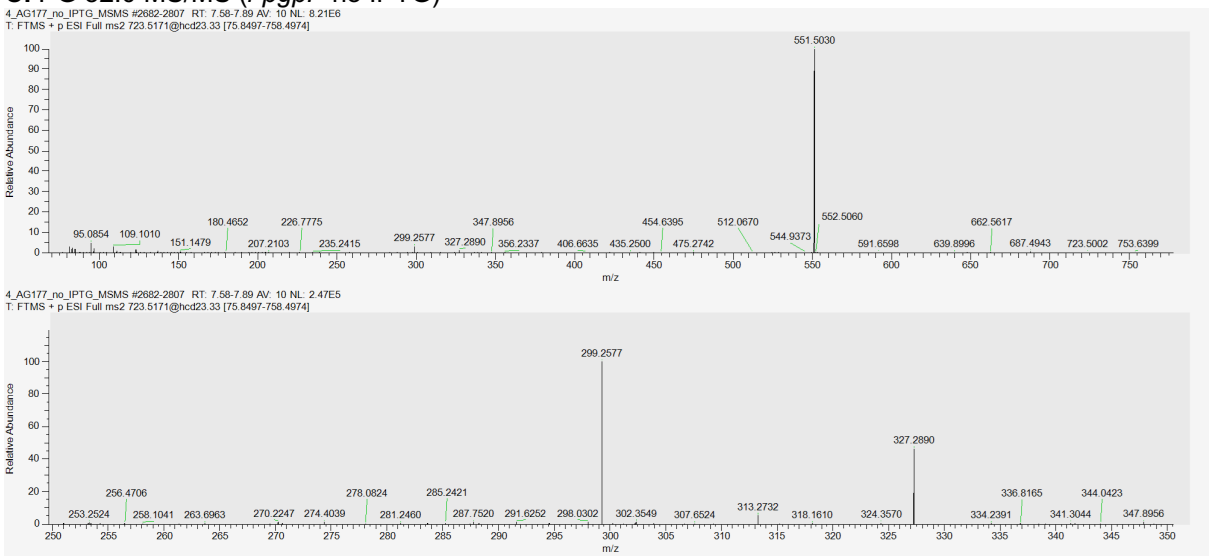
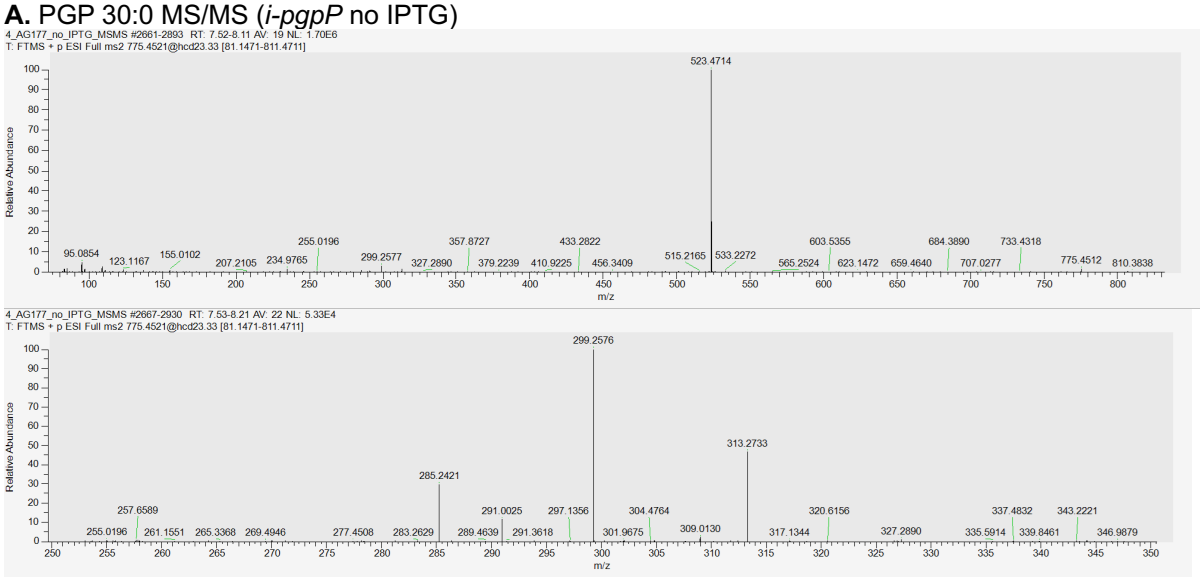
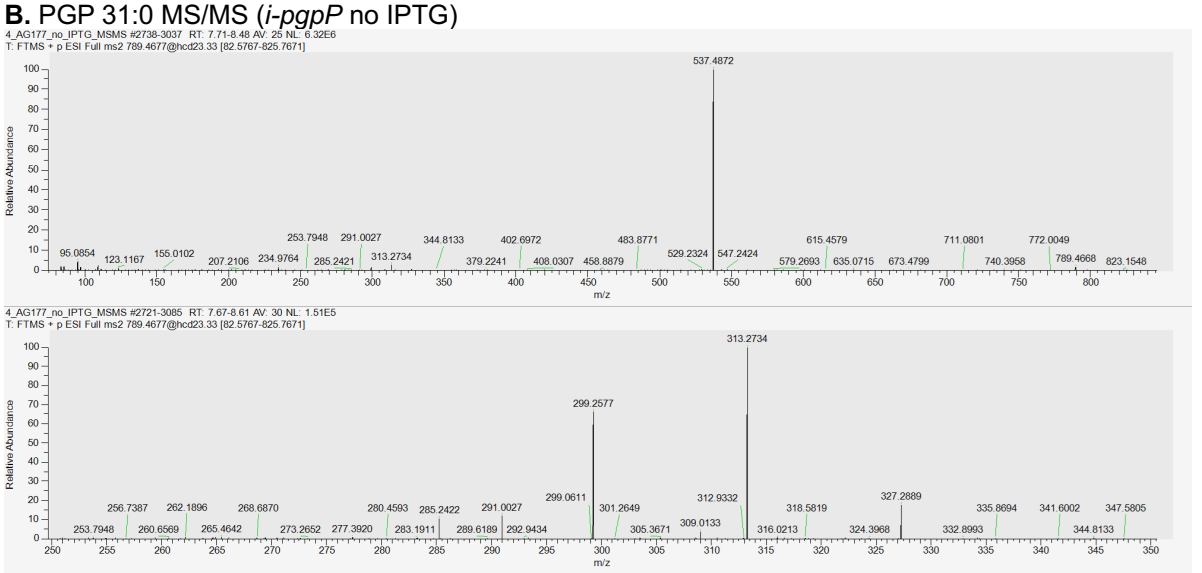


Figure S17. MS/MS spectra for (A) PG 30:0, (B) PG 30:1, (C) PG 32:0 lipids isolated from strain PY79 *i-pgpP* grown in the absence of IPTG. Top panels are full range spectra showing the mass signal for Fragment 1 (loss of head group) and bottom panels are zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

A. PGP 30:0 MS/MS (*i-pgpP* no IPTG)



B. PGP 31:0 MS/MS (*i-pgpP* no IPTG)



C. PGP 32:0 MS/MS (*i-pgpP* no IPTG)

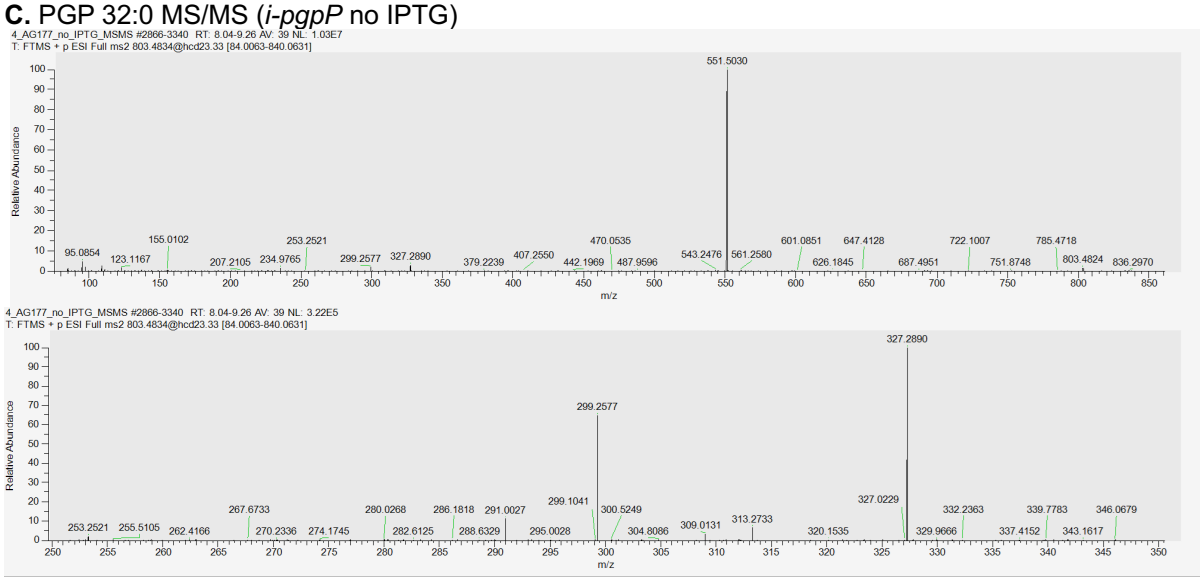


Figure S18. MS/MS spectra for (A) PGP 30:0, (B) PGP 30:1, (C) PGP 32:0 lipids isolated from strain PY79 *i-pgpP* grown in the absence of IPTG. Top panels are full range spectra showing the mass signal for Fragment 1 (loss of head group) and bottom panels are zoomed in view of the 250-350 mass range showing Fragments 2 (a and b) peaks (loss of head group and one fatty acid).

Supporting Tables

Table S1. DELTA_BLAST search results using *E. coli* Pgp enzymes as query sequences against the *B. subtilis* genome

Query Sequence	*B. subtilis* 168 Locus Tag	Accession	Max Score	Total Score	Query Coverage	E-Value	% Identify	Protein length (AA)
E. coli PgpA	BSU_32280 (YutG)	WP_003243814.1	108	108	90%	3e-31	14.11	166
	BSU_21830 (YpjQ)	WP_003230797.1	89.4	89.4	83%	1e-23	16.56	177
E. coli PgpB	BSU_19650 (PgpB/YodM)	WP_004399336.1	68.9	68.9	71%	5e-15	17.65	203
	BSU_36530 (BcrC)	WP_003243362.1	64.7	64.7	77%	2e-13	14.72	193
E. coli PgpC	BSU_33500 (CopA)	WP_003242925.1	63.9	63.9	94%	8e-13	11.84	802
	BSU_33490 (CadA)	WP_014906546.1	60.1	60.1	97%	2e-11	10.33	699
	BSU_13850 (PfeT)	WP_003245873.1	58.2	58.2	90%	8e-11	11.86	637
	BSU_14550 (YkrA)	WP_003245153.1	53.9	53.9	98%	1e-09	10.73	257
	BSU_13600 (MtnX)	WP_003245748.1	53.5	53.5	94%	2e-09	10.10	235
	BSU_34550 (PgcM)	WP_003228210.1	50.1	50.1	64%	2e-08	11.68	226
	BSU_34970 (YvoE)	WP_003242685.1	48.5	48.5	94%	8e-08	11.00	216
	BSU_09240 (YhcW)	WP_003245030.1	46.2	46.2	86%	4e-07	9.68	220
	BSU_35850 (YwtE)	WP_003244395.1	45.8	45.8	18%	8e-07	19.51	286
	BSU_04040 (YcsE)	WP_003234424.1	45.1	45.1	92%	1e-06	12.39	249
	BSU_36290 (PhoC/YwpJ)	WP_003242946.1	44.7	76.2	72%	2e-06	19.44	285
	BSU_10540 (NtdB)	WP_003244845.1	42.4	42.4	36%	1e-05	14.81	282
	BSU_15650 (YloB)	WP_003232087.1	42.4	42.4	43%	2e-05	12.87	890
	BSU_28940 (SerB)	WP_004398978.1	40.4	40.4	95%	4e-05	10.29	260
	BSU_07330 (YfnB)	WP_003244307.1	39.7	39.7	93%	9e-05	13.59	235
	BSU_11140 (YitU)	WP_003233007.1	35.8	35.8	17%	0.002	16.67	270
	BSU_09830 (YhaX)	WP_003233277.1	34.7	34.7	36%	0.004	6.49	288

Table S2. Chemical formula and exact masses of the control PG lipid and *B. subtilis* PG and PGP lipids

Lipid	Formula	Mass	Formula +H	Mass + H
PG 15:0 18:1 D7	C39H68D7O10P	741.5537	C39H69D7O10P	742.5615
PG 30:0	C36H71O10P	694.4785	C36H72O10P	695.4863
PG 31:0	C37H73O10P	708.4941	C37H74O10P	709.5020
PG 32:0	C38H75O10P	722.5098	C38H76O10P	723.5176
PGP 30:0	C36H72O13P2	774.4448	C36H73O13P2	775.4526
PGP 31:0	C37H74O13P2	788.4605	C37H75O13P2	789.4683
PGP 32:0	C38H76O13P2	802.4761	C38H77O13P2	803.4839

Table S3. Table with chemical formulas, expected and observed masses for PG and PGP lipid MS/MS fragments

		Formula + H	Expected Mass + H	Observed Mass + H	Fatty acid chain
Control lipid					
PG C15:0 C18:1-D7	Fragment 1	C36H60D7O4	570.5479	570.5473	C15:0 / C18:1-D7
	Fragment 2a	C18H35O3	299.2586	299.2581	C15:0
	Fragment 2b	C21H32D7O3	346.3339	346.3333	C18:1-D7
WT with IPTG					
PG 30:0	Fragment 1	C33H63O4	523.4726	523.4714	C15:0 / C15:0
	Fragment 2a / 2b	C18H35O3	299.2586	299.2575	C15:0
PG 31:0	Fragment 1	C34H65O4	537.4883	537.4870	C15:0 / C16:0
	Fragment 2a	C18H35O3	299.2586	299.2576	C15:0
	Fragment 2b	C19H37O3	313.2743	313.2733	C16:0
PG 32:0	Fragment 1	C35H67O4	551.5039	551.5028	C15:0 / C17:0
	Fragment 2a	C18H35O3	299.2586	299.2577	C15:0
	Fragment 2b	C20H39O3	327.2899	327.2888	C17:0
PGP 30:0	Fragment 1	C33H63O4	523.4726	523.4713	C15:0 / C15:0
	Fragment 2a/ 2b	C18H35O3	299.2586	299.2576	C15:0
PGP 31:0	Fragment 1	C34H65O4	537.4883	537.4872	C15:0 / C16:0
	Fragment 2a	C18H35O3	299.2586	299.2575	C15:0
	Fragment 2b	C19H37O3	313.2743	313.2731	C16:0
PGP 32:0	Fragment 1	C35H67O4	551.5039	551.5027	C15:0 / C17:0
	Fragment 2a	C18H35O3	299.2586	299.2575	C15:0
	Fragment 2b	C20H39O3	327.2899	327.2888	C17:0
WT no IPTG					
PG 30:0	Fragment 1	C33H63O4	523.4726	523.4713	C15:0 / C15:0
	Fragment 2a / 2b	C18H35O3	299.2586	299.2577	C15:0
PG 31:0	Fragment 1	C34H65O4	537.4883	537.4870	C15:0 / C16:0
	Fragment 2a	C18H35O3	299.2586	299.2576	C15:0
	Fragment 2b	C19H37O3	313.2743	313.2732	C16:0
PG 32:0	Fragment 1	C35H67O4	551.5039	551.5028	C15:0 / C17:0
	Fragment 2a	C18H35O3	299.2586	299.2576	C15:0
	Fragment 2b	C20H39O3	327.2899	327.2889	C17:0
PGP 30:0	Fragment 1	C33H63O4	523.4726	523.4717	C15:0 / C15:0
	Fragment 2a/ 2b	C18H35O3	299.2586	299.2575	C15:0
PGP 31:0	Fragment 1	C34H65O4	537.4883	537.4870	C15:0 / C16:0
	Fragment 2a	C18H35O3	299.2586	299.2577	C15:0
	Fragment 2b	C19H37O3	313.2743	313.2734	C16:0
PGP 32:0	Fragment 1	C35H67O4	551.5039	551.5030	C15:0 / C17:0
	Fragment 2a	C18H35O3	299.2586	299.2576	C15:0
	Fragment 2b	C20H39O3	327.2899	327.2889	C17:0
***i-pgpP* with IPTG**					
PG 30:0	Fragment 1	C33H63O4	523.4726	523.4717	C15:0 / C15:0
	Fragment 2a / 2b	C18H35O3	299.2586	299.2577	C15:0
PG 31:0	Fragment 1	C34H65O4	537.4883	537.4873	C15:0 / C16:0
	Fragment 2a	C18H35O3	299.2586	299.2575	C15:0
	Fragment 2b	C19H37O3	313.2743	313.2733	C16:0
PG 32:0	Fragment 1	C35H67O4	551.5039	551.5030	C15:0 / C17:0
	Fragment 2a	C18H35O3	299.2586	299.2577	C15:0
	Fragment 2b	C20H39O3	327.2899	327.2889	C17:0
PGP 30:0	Fragment 1	C33H63O4	523.4726	523.4716	C15:0 / C15:0
	Fragment 2a/ 2b	C18H35O3	299.2586	299.2578	C15:0
PGP 31:0	Fragment 1	C34H65O4	537.4883	537.4872	C15:0 / C16:0
	Fragment 2a	C18H35O3	299.2586	299.2576	C15:0
	Fragment 2b	C19H37O3	313.2743	313.2734	C16:0
PGP 32:0	Fragment 1	C35H67O4	551.5039	551.5030	C15:0 / C17:0
	Fragment 2a	C18H35O3	299.2586	299.2576	C15:0
	Fragment 2b	C20H39O3	327.2899	327.2889	C17:0
***i-pgpP* no IPTG**					
PG 30:0	Fragment 1	C33H63O4	523.4726	523.4713	C15:0 / C15:0
	Fragment 2a / 2b	C18H35O3	299.2586	299.2577	C15:0
PG 31:0	Fragment 1	C34H65O4	537.4883	537.4874	C15:0 / C16:0
	Fragment 2a	C18H35O3	299.2586	299.2576	C15:0
	Fragment 2b	C19H37O3	313.2743	313.2734	C16:0
PG 32:0	Fragment 1	C35H67O4	551.5039	551.5030	C15:0 / C17:0
	Fragment 2a	C18H35O3	299.2586	299.2577	C15:0

	Fragment 2b	C20H39O3	327.2899	327.2890	C17:0
PGP 30:0	Fragment 1	C33H63O4	523.4726	523.4714	C15:0 / C15:0
	Fragment 2a/ 2b	C18H35O3	299.2586	299.2576	C15:0
PGP 31:0	Fragment 1	C34H65O4	537.4883	537.4872	C15:0 / C16:0
	Fragment 2a	C18H35O3	299.2586	299.2577	C15:0
	Fragment 2b	C19H37O3	313.2743	313.2734	C16:0
PGP 32:0	Fragment 1	C35H67O4	551.5039	551.5030	C15:0 / C17:0
	Fragment 2a	C18H35O3	299.2586	299.2577	C15:0
	Fragment 2b	C20H39O3	327.2899	327.2890	C17:0

Table S4. Distribution of PgsA (COG0558), PgpA (COG1267) and PgpP (YqeG) (COG02179) in Archaea and bacteria listed in the COG database.

KINGDOM	PHYLUM	CLASS	Nr of strains	PgsA (COG0558)	PgpA (COG1267)	PgpP (YqeG) (COG02179)
ARCHAEA	CRENARCHAEOTA		25	23	7	0
	EURYARCHAEOTA		79	74	31	0
	THAUMARCHAEOTA		12	12	0	0
	OTHER ARCHAEA		6	3	0	0
BACTERIA	ACIDOBACTERIA		7	7	7	0
	ACTINOBACTERIA		155	155	0	10*
	AQUIFICAE		9	9	9	0
	BACTEROIDETES		107	36	11	0
	CHLAMYDIAE		6	6	0	0
	CHLOROBI		5	5	5	0
	CHLOROFLEXI		14	14	0	0
	CYANOBACTERIA		41	41	0	41
	DEFERRIBACTERES		5	5	0	0
	DEINOCOCCUS-THERMUS		6	1	0	6
	FIRMICUTES	BACILLI	73	73	55	72
		CLOSTRIDIA	79	72	15	60
		NEGATIVICUTES	10	0	1	3
		TISSIERELLIA	9	9	4	0
		OTHER FIRMICUTES	4	4	3	2
	FUSOBACTERIA		6	6	2	0
	MOLLICUTES		14	14	3	9
	PLANCTOMYCETES		14	14	5	1
	PROTEOBACTERIA	ALPHAPROTEOBACTERIA	158	156	69	0
		BETAPROTEOBACTERIA	102	97	95	0
		DELTAPROTEOBACTERIA	39	39	26	1
		EPSILONPROTEOBACTERIA	12	12	12	0
		GAMMAPROTEOBACTERIA	224	217	178	7
		OTHER PROTEOBACTERIA	6	6	6	0
	SPIROCHAETES		11	11	1	0
	SYNERGISTETES		5	5	5	0
	THERMOTOGAE		9	9	0	9
	VERRUCOMICROBIA		9	9	6	0
	OTHER BACTERIA		48	30	18	5

* PgpP (YqeG)-type proteins are only found in Coriobacteriia within the Actinobacteria

Table S5. Bacterial strains used in this study

Unique ID	Strain description	Reference
E. coli		
ANG127	XL1-Blue; Tet^R	Stratagene
ANG2111/AG022	YL24 pMAK-C; Kan^R, Cam^R, 0.2% arabinose, 30°C	(3)
AG118	MG1655 (WT *E. coli*)	(4)
AG107/cWX856	DH5-α pWX466 (Spec Marker); Amp^R	(5)
AG109/cWX860	DH5-α pWX470 (Kan Marker); Amp^R	(5, 6)
AG111/cWX867	DH5-α pWX469 (Tet Marker); Amp^R	(7)
AG110/cWX865	DH5-α pWX467 (MLS Marker); Amp^R	(5)
AG113/cDR105	TG1 pDR244 (pCre-spec plasmid); Amp^R	(7)
AG114/cWX950	TG1 pWX493 (pCre-erm plasmid); Amp^R	(5)
AG125/cWX1277	TG1 pWX642 (transposon delivery plasmid); Amp^R	(8)
AG143/cDR2113	DH5-α pCB095 (*PxylA ycgO::erm*); Amp^R	Rudner Lab
AG156/cDR2037	DH5-α pCB059 (*Pspank yhdG::kan*); Amp^R	(9)
AG174	XL1-Blue pCB059-$pgpP_{BS}$; Amp^R	This study
AG315	XL1-Blue pCB095-$pgpA_{EC}$; Amp^R	This study
AG316	XL1-Blue pCB095-$pgpC_{EC}$; Amp^R	This study
AG317	XL1-Blue pCB095-$pgpP_{SA}$; Amp^R	This study
AG318	XL1-Blue pCB095-$pgpP_{BS}$; Amp^R	This study
AG319	XL1-Blue pCB095-*pgpP-His*; Amp^R	This study
AG341	XL1-Blue pCB095-$pgpP_{D34N}$-*His*; Amp^R	This study
AG342	XL1-Blue pCB095-$pgpP_{D36N}$-*His*; Amp^R	This study
AG355	YL24 pMAK-C pCB095; Kan^R, Cam^R, 0.2% arabinose, 30°C	This study
AG357	YL24 pMAK-C pCB095-$pgpA_{EC}$; Kan^R, Cam^R, 0.2% arabinose, 30°C	This study
AG359	YL24 pMAK-C pCB095-$pgpC_{EC}$; Kan^R, Cam^R, 0.2% arabinose, 30°C	This study
AG361	YL24 pMAK-C pCB095-$pgpP_{SA}$; Kan^R, Cam^R, 0.2% arabinose, 30°C	This study
AG363	YL24 pMAK-C pCB095-$pgpP_{BS}$; Kan^R, Cam^R, 0.2% arabinose, 30°C	This study
ANG1268	XL1-Blue pUT18C	(10)
CIR469	DH5-α pIR469 [*ycgO::Pspank-pgsA(specR)*]; Amp^R	This study
B. subtilis		
AG101/bDR11	PY79 (WT)	(11, 12)
AG121/bIR636	PY79 *bcrC::erm*; MLS^R	(13)
AG159/bDR2259	PY79 *yhdG::spec*; $Spec^R$	Rudner Lab
AG160/bKM400	PY79 *ycgO::cat*; Cam^R	Rudner Lab
AG176	PY79 *yhdG::Pspank-*$pgpP_{BS}$ *(kan)*; Kan^R	This study
AG177	PY79 *yhdG::Pspank-*$pgpP_{BS}$ *(kan)* Δ*pgpP::spec* (**PY79 *i-pgpP***); Kan^R, $Spec^R$, IPTG	This study
AG191/bDR2789	PY79 *sacA::Pveg-GFP* (phleo) (PY79 GFP); $Phleo^R$	(13)
AG195	PY79 *yhdG::Pspank-*$pgpP_{BS}$ *(kan)* Δ*pgpP::spec,* sacA::Pveg-GFP (phleo) (**PY79 *i-pgpP GFP***); Kan^R, $Spec^R$, $Phleo^R$, IPTG	This study
AG201	PY79 Δ*ypjQ::tet*; Tet^R	This study
AG202	PY79 Δ*yutG::erm*; MLS^R	This study
AG203	PY79 Δ*pgpB::kan*; Kan^R	This study
AG208	PY79 Δ*ypjQ::tet yutG::erm*; Tet^R, MLS^R	This study

AG209	PY79 Δ*ypjQ::tet,* Δ*yutG::erm,* Δ*pgpB::kan*, Tet^R, MLS^R, Kan^R	This study
AG216	PY79 Δ*ypjQ::lox72* Δ*yutG::lox72*	This study
AG217	PY79 Δ*ypjQ::lox72,* Δ*yutG::lox72,* Δ*pgpB::lox72*	This study
AG228	PY79 Δ*pgpB::lox72*	This study
AG233	PY79 Δ*ypjQ::lox72,* Δ*yutG::lox72,* Δ*pgpB::lox72,* Δ*bcrC::erm*; MLS^R	This study
AG240	PY79 Δ*ypjQ::lox72,* Δ*yutG::lox72,* Δ*pgpB::lox72,* Δ*bcrC::lox72*	This study
AG289	PY79 Δ*bcrC::lox72*	This study
AG290	PY79 Δ*bcrC::erm*; MLS^R	This study
AG303	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec* (**PY79 *i-pgpP ycgO::cat***); Kan^R, Spec^R, Cam^R, IPTG	This study
AG321	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpA$_{EC}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpA$_{EC}$***); Kan^R, Spec^R, MLS^R, IPTG	This study
AG323	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpC$_{EC}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpC$_{EC}$***); Kan^R, Spec^R, MLS^R, IPTG	This study
AG325	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpP$_{SA}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpP$_{SA}$***); Kan^R, Spec^R, MLS^R, IPTG	This study
AG327	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpP$_{BS}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpP$_{BS}$***); Kan^R, Spec^R, MLS^R, IPTG	This study
AG329	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO:: PxylA-pgpP-His* (*erm*) (**PY79 *i-pgpP Pxyl-pgpP-His***); Kan^R, Spec^R, MLS^R, IPTG	This study
AG343	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::Pxyl (erm)* (**PY79 *i-pgpP* (EV)**); Kan^R, Spec^R, MLS^R, IPTG	This study
AG345	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO:: PxylA-pgpP$_{D34N}$-His* (*erm*) (**PY79 *i-pgpP Pxyl-pgpP$_{D34N}$-His***); Kan^R, Spec^R, MLS^R, IPTG	This study
AG347	PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO:: PxylA-pgpP$_{D36N}$-His* (*erm*) (**PY79 *i-pgpP Pxyl-pgpP$_{D36N}$-His***); Kan^R, Spec^R, MLS^R, IPTG	This study
AG371/BIR1527	*PY79 ycgO::Pspank-pgsA (spec) Spec^R*	This study
AG375/BIR1532	PY79 *ycgO::Pspank-pgsA(spec)* Δ*pgsA::erm* (**PY79 *i-pgsA***); Spec^R, MLS^R, IPTG	This study
S. aureus		
ANG113	RN4220	(14)
ANG1517	LAC*	(15)

Table S6. Primers and oligos used in this study

Primer ID	Sequence
oAB114	CTGAGCGAGGGAGCAGAA
oAL23	TTCAGAACGCTCGGTTGCC
oAL195	ATCCCTACAGTGTTATGGCTTGAACAA
oANG1	CGTGAGTATTTTACTGGCCAGGAGC
oANG2	AATTCGATCTGATGAGAGGCCTTCC
oANG3	AACGAGGGCTATAGAACTGACAGAC
oANG11	CAAAAACAAAGGCCAAGCTTAAATCG
oANG14	CTTCTTTTAAAAGTGCCGTCTGTATTGG
oANG15	CGGTACTGAGCGAGGGAGCAGAATACTTTTTCATGTCTAATGGCATCTCC
oANG16	CGGTAGTTGACCAGTGCTCCCTGGAAAAGCCTGTCGTCAGCTGAC
oANG17	CGGTACTGAGCGAGGGAGCAGAATTTTCTGACATCAGCTTCACCCAATC
oANG18	CGGTAGTTGACCAGTGCTCCCTGCATACAGAATAAGGGCACCCTTTTAGG
oANG19	CGGTACTGAGCGAGGGAGCAGAAGGCTTGTACAATTTTAACCACCTC
oANG20	CGGTAGTTGACCAGTGCTCCCTGCGACAAAAATAGAGAGAGGAAAACAC
oANG25	CGGTACTGAGCGAGGGAGCAGAATTTTTTAACAAAACCCGCACCCTTC
oANG26	CGGTAGTTGACCAGTGCTCCCTGAAACGAAAAGGGCACATTCAGTG
oANG36	TAACAATTAAGCTTGTCCCGGGTAACTAGTAAAAGGGAAGGGTGCGGGTTTTGTTAAAAAAG
oANG37	GCTTGCATGCGAGCTAGCATCTGCAGTTACTCCTCCCACTGAATGTGCCCT
oANG52	ATCTCGAGACATAAGGAGGAACTACTATGACCATTTTGCCACGCCATAAAG
oANG53	CTGGATCCCTACGACAGAATACCCAGCGGC
oANG54	GCATCTCGAGACATAAGGAGGAACTACTTTGGCAACTCACGAGCGTCGTG
oANG55	CTGGATCCCTATTCCAGTTGCTGGAGTTCACCG
oANG56	GCATCTCGAGACATAAGGAGGAACTACTATGGGTTTAGTTCGCAAGTTTTTTATGC
oANG57	CTGGATCCTCAATTTTCCTCCCATGTGATATAACCTTTTTTAC
oANG58	GCATCTCGAGAAAAGGGAAGGGTGCGGGTTTTG
oANG59	CTGGATCCTTACTCCTCCCACTGAATGTGCCC
oANG60	GTAAAAGGAATTATTACTAACCTGGATAATACGCTTGTTG
oANG61	CAACAAGCGTATTATCCAGGTTAGTAATAATTCCTTTTAC
oANG62	GGAATTATTACTGACCTGAATAATACGCTTGTTGAATG
oANG63	CATTCAACAAGCGTATTATTCAGGTCAGTAATAATTCC
oANG64	ATGGTGGTGGTGATGAGAGCCGCTGCCACTAGTCTCCTCCCACTGAATGTGCCCTTTTCG
oANG65	AAGGATCCTTAATCAATTAATGGTGATGGTGGTGATGGTGGTGGTGATGAGAGCCGCTGC
oANG66	ATCAACATAGTACATAGCGAATCTTCCC
oANG67	TTTATAGTTTTGGTCGTAGAGCACACGG
oANG68	ATGAAGCTTGAGCTAGCATCTCGAGACATAAGGAGGAACTACTATG
oANG69	GAGGGTTGCCAGAGTTAAAGGATCCCTACGACAGAATACCCAG
oANG70	ATGAAGCTTGAGCTAGCATCTCGAGACATAAGGAGGAACTACTTTG
oANG71	GAGGGTTGCCAGAGTTAAAGGATCCCTATTCCAGTTGCTGGAG
oANG72	GAGGGTTGCCAGAGTTAAAGGATCCTCAATTTTCCTCCCATGTG
oANG73	ATGAAGCTTGAGCTAGCATCTCGAGAAAAGGGAAGGGTGCGGG
oANG74	GAGGGTTGCCAGAGTTAAAGGATCCTTACTCCTCCCACTGAATGTG
oANG75	GAGGGTTGCCAGAGTTAAAGGATCCTTAATCAATTAATGGTGATGGTGGTGATG
oANG18_ABG	GCCTGTTCGACGATGGGCTG
oANG19_ABG	CGTCAGGGCGCGTCAGCGGG
oJM028	TTCTGCTCCCTCGCTCAG
oJM029	CAGGGAGCACTGGTCAAC
oIR1109	CATTTTATTCCGCGTCATGAAATTG
oIR1112	CGATAAAAGGCAAGCGGAACAC
oIR1113	CAACACTGGCTTCAAAGTAGATATC
oIR1116	TGCGGTCCGACACATGAATG
oIR1117	CTGCGGTATGACCTATCAAGAGG

oIR1120	CATATTGTTTATTGACAAGCGCGAG
oIR1381	ACAATTAAGCTTCTTCACGTCCGAGTATGAAATTGGAGG
oIR1382	TGCAGTTACTAGTTAGTTAGATGTTTTTAACGCTTCCC
oIR1383	GACTGCTCGGGGATATTTCATCTC
oIR1384	CGGTACTGAGCGAGGGAGCAGAAAAAGCCCCTCCAATTTCATACTC
oIR1385	CGGTAGTTGACCAGTGCTCCCTGGGGAAGCGTTAAAAACATCTAAC
oIR1386	GTGTCAGGCTGAGACAGTAAAAGC
oYY30	CGCCGGCTGGATCTTAGTAGA
oYY31	CTCTGCTTCTCTGAGCGGTAC

Supporting Text

Construction of bacterial strains and plasmids used in this study.

***E. coli* strain and plasmid construction:**

AG174
XL1-Blue pCB059-*pgpP$_{BS}$*
Plasmid pCB059-*pgpP$_{BS}$* was constructed by Gibson assembly. To this end, the *ygeG$_{BS}$* gene was amplified by PCR using primers oAG36/oAG37 and introducing an optimized RBS sequence as part of the forward primer and using *B. subtilis* PY79 gDNA (strain AG101) as template. The cleaned-up PCR fragment was Gibson assembly into plasmid pCB059 (strain AG156) cut with SpeI and PstI and recovered in the *E. coli* strain XL1 Blue (ANG127). The sequence of the insert was confirmed by sanger sequencing using primers oAL23 and oAL195. The plasmid pCB059-*pgpP$_{BS}$* can be used for integration of a Kan marked *Pspank-pgpP$_{BS}$* fragment into the *yhdG* gene in *B. subtilis.*

AG315
XL1-Blue pCB095-*pgpA$_{EC}$*
Plasmid pCB095-*pgpA$_{EC}$* was constructed by Gibson assembly and can be used for xylose inducible *pgpA$_{EC}$* expression. The *pgpA$_{EC}$* gene was first amplified using primers oANG52/oANG53 and MG1655 gDNA (AG118) as template and re-amplified using primers oANG68/oANG69. The cleaned-up PCR fragment was Gibson assembly into pCB095 (AG143) cut with XhoI and BamHI and the sequence of the insert was confirmed by sanger sequencing using primers oANG66/oANG67. The plasmid pCB095-*pgpA$_{EC}$* can be used for integration of an MLS marked *Pxyl-pgpA$_{EC}$* fragment into the *ycgO* gene in *B. subtilis.*

AG316
XL1-Blue pCB095-*pgpC$_{EC}$*
Plasmid pCB095-*pgpC$_{EC}$* was constructed by Gibson assembly and can be used for xylose inducible *pgpC$_{EC}$* expression. The *pgpC$_{EC}$* gene was first amplified using primers oANG54/oANG55 and MG1655 gDNA (AG118) as template and re-amplified using primers oANG70/oANG71. The cleaned-up PCR fragment was Gibson assembled into pCB095 (AG143) cut with XhoI and BamHI and the sequence of the insert was confirmed by sanger sequencing using primers oANG66/oANG67. The plasmid pCB095-*pgpC$_{EC}$* can be used for integration of an MLS marked *Pxyl-pgpC$_{EC}$* fragment into the *ycgO* gene in *B. subtilis.*

AG317
XL1-Blue pCB095-*pgpP$_{SA}$*
Plasmid pCB095-*pgpP$_{SA}$* was constructed by Gibson assembly and can be used for xylose inducible *pgpP$_{SA}$* expression. The *pgpP$_{SA}$* gene was first amplified using primers oANG56/oANG57 and LAC* gDNA (AG123) as template and re-amplified using primers oANG68/oANG7. The cleaned-up PCR fragment was Gibson assembly into pCB095 (AG143) cut with XhoI and BamHI and the sequence of the insert was confirmed by sanger sequencing using primers oANG66/oANG67. The plasmid pCB095-*pgpP$_{SA}$* can be used for integration of an MLS marked *Pxyl-pgpP$_{SA}$* fragment into the *ycgO* gene in *B. subtilis.*

AG318
XL1-Blue pCB095-*pgpP$_{BS}$*
Plasmid pCB095-*pgpP$_{BS}$* was constructed by Gibson assembly and can be used for xylose inducible *pgpP$_{BS}$* expression. The *pgpP$_{BS}$* gene was first amplified using primers oANG58/oANG59 and PY79 gDNA (AG101) as template and re-amplified using primers oANG73/oANG74. The cleaned-up PCR fragment was Gibson assembled into pCB095 (AG143) cut with XhoI and BamHI and the sequence of the insert was confirmed by sanger sequencing using primers oANG66/oANG67. The plasmid pCB095-*pgpP$_{BS}$* can be used for integration of an MLS marked *Pxyl-pgpP$_{BS}$* fragment into the *ycgO* gene in *B. subtilis.*

AG319
XL1-Blue pCB095-*pgpP-His*

Plasmid pCB095-*pgpP-His* was constructed by Gibson assembly and can be used for xylose inducible *pgpP-His* expression. The $pgpP_{BS}$ gene was first amplified using primers oANG58/oANG64 and PY79 gDNA (AG101) as template and part of the C-terminal His tag was added as part of the reverse primer. The PCR fragment was re-amplified in a second PCR with primers oANG58/oANG65 adding the complete C-terminal His tag. The cleaned-up PCR product was finally amplified with primers oANG73/oANG75 to add plasmid overhands and Gibson assembled into pCB095 (AG143) cut with XhoI and BamHI. The sequence of the insert was confirmed by sanger sequencing using primers oANG66/oANG67. The plasmid pCB095-*pgpP-His* can be used for integration of an MLS marked *Pxyl-pgpP-His* fragment into the *ycgO* gene in *B. subtilis.*

AG341
XL1-Blue pCB095-$pgpP_{D34N}$-*His*
Plasmid pCB095- $pgpP_{D34N}$-*His* was constructed by splicing overhang extension (SOE) PCR and can be used for xylose inducible $pgpP_{D34N}$-*His* expression. Using plasmid pCB095-*pgpP-His* (AG319) as template, the front part of the gene was amplified with primers oANG73/oANG61 and the back part with primers oANG60/oANG75 introducing the point mutation with the internal primers. The reaction was incubated with DpnI to remove the template plasmid, and the PCR fragments gel extract and fused together in a second PCR using primers oANG73/oANG75. The cleaned-up PCR fragment was Gibson assembled into plasmid pCB095 (AG143), which had been cut with enzymes XhoI and BamHI. The sequence of the insert was confirmed by sanger sequencing using primers oANG66/oANG67. The plasmid pCB095-$pgpP_{D34N}$-*His* can be used for integration of an MLS marked *Pxyl-*$pgpP_{D34N}$-*His* fragment into the *ycgO* gene in *B. subtilis.*

AG342
XL1-Blue pCB095-$pgpP_{D36N}$-*His*
Plasmid pCB095-*pgpP-His* was constructed by SOE PCR and can be used for xylose inducible $pgpP_{D36N}$-*His* expression. Using plasmid pCB095-*pgpP-His* (AG319) as template, the front part of the gene was amplified with primers oANG73/oANG63 and the back part with primers oANG62/oANG75 introducing the point mutation with the internal primers. The reaction was incubated with DpnI to remove the template plasmid, and the PCR fragments gel extract and fused together in a second PCR using primers oANG73/oANG75. The cleaned-up PCR fragment was Gibson assembles into plasmid pCB095 (AG143), which had been cut with enzymes XhoI and BamHI. The sequence of the insert was confirmed by sanger sequencing using primers oANG66/oANG67. The plasmid pCB095-$pgpP_{D36N}$-*His* can be used for integration of an MLS marked *Pxyl-*$pgpP_{D36N}$-*His* fragment into the *ycgO* gene in *B. subtilis.*

AG355
YL24 pMAK-C pCB095 (EV)
Plasmid pCB095 from strain AG143 was introduced into the temperature sensitive *pgp* gene depletion *E. coli* strain YL24 pMAK-C (AG022). Colonies were recovered on LB Amp100/Kan30/Cam10 0.2% arabinose plates at 30°C. This is the empty vector control strain.

AG357
YL24 pMAK-C pCB095-$pgpA_{EC}$
Plasmid pCB095-$pgpA_{EC}$ from strain AG315 was introduced into the temperature sensitive *pgp* gene depletion *E. coli* strain YL24 pMAK-C (AG022). Colonies were recovered on LB Amp100/Kan30/Cam10 0.2% arabinose plates at 30°C.

AG359
YL24 pMAK-C pCB095-$pgpC_{EC}$
Plasmid pCB095-$pgpC_{EC}$ from strain AG316 was introduced into the temperature sensitive *pgp* gene depletion *E. coli* strain YL24 pMAK-C (AG022). Colonies were recovered on LB Amp100/Kan30/Cam10 0.2% arabinose plates at 30°C.

AG361
YL24 pMAK-C pCB095-$pgpP_{SA}$

Plasmid pCB095-*pgpP$_{SA}$* from strain AG317 was introduced into the temperature sensitive *pgp* gene depletion *E. coli* strain YL24 pMAK-C (AG022). Colonies were recovered on LB Amp100/Kan30/Cam10 0.2% arabinose plates at 30°C.

AG363
YL24 pMAK-C pCB095-*pgpP$_{BS}$*
Plasmid pCB095-*pgpP$_{BS}$* from strain AG318 was introduced into the temperature sensitive *pgp* gene depletion *E. coli* strain YL24 pMAK-C (AG022). Colonies were recovered on LB Amp100/Kan30/Cam10 0.2% arabinose plates at 30°C.

CIR469
DH5-α pIR469 [*ycgO::Pspank-pgsA(specR)*]
For construction of plasmid pIR469 the *pgsA* gene was amplified by PCR with primers oIR1381 and oIR1382 and using PY79 gDNA as template. The PCR product was digested with HindIII and SpeI and ligated with plasmid pCB084 and in this manner placing the *pgsA* gene under the control of the *Pspank* promoter.

B. subtilis strain construction

AG176
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)*
This strain was constructed by transforming strain PY79 *yhdG::spec* (AG159), which contains a spec marker in the *yhdG* gene, with ScaI linearized pCB059-*pgpP$_{BS}$* plasmid isolated from *E. coli* strain AG174. In this manner the spec marker in the *yhdG* gene was replaced with the *yhdG::spank-pgpP$_{BS}$ (kan)* fragment for IPTG inducible *pgpP* expression. This strain still contains a WT copy of the *pgpP* gene at the native locus.

AG177
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec* (PY79 *i-pgpP*)
This is a *B. subtilis* strain for IPTG-inducible *pgpP$_{BS}$* expression from the spank promoter. The *B. subtilis* strain PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* (strain AG176) was transformed with a Gibson assembled *pgpP::spec* fragment to replace the *pgpP* gene at the native locus with a Spec marker. For generation of the Gibson assembled fragment a *loxP*-site flaked *spec* marker was amplified with primers oJM028/oJM029 from plasmid pWX466 (strain AG107) and assembled with an approximately 1.8 kb upstream *pgpP* fragment generated by PCR using primers oANG11/oANG25 and an approximately 1.8 kb downstream fragment generated by PCR using primers oANG26/oANG14 and using PY79 (AG101) gDNA as template. The three fragments were assembled in a Gibson reaction and transform into strain AG176. Transformants were selected on Spec100 plates in the presence of 250 - 500 µM IPTG.

AG195
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec, sacA::Pveg-GFP* (phleo) (PY79 *i-pgpP GFP*)
Strain PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec* (PY79 *i-pgpP*) (AG177) was transformed with gDNA isolated from strain PY79 *sacA::Pveg-GFP* (phleo) (AG191) and transformants selected on Phleo plates containing 250 - 500 µM IPTG.

AG201
PY79 *ypjQ::tet*
PY79 *ypjQ::tet* mutant strain was constructed by transforming a Gibson assembled *ypjQ::tet* fragment into WT *B. subtilis* strain PY79 (BDR11/AG101). For generation of the Gibson assembled fragment a ca. 1.8 kb upstream *ypjQ* fragment was amplified with primers oIR1109/oANG15 and a ca. 1.8kb downstream *ypjQ* fragment using primers oANG16/oIR1112. The *loxP* site flanked *tet* marker was amplified using plasmid pWX469 from strain cWX867 (AG111) as templet and primers oJM028/oJM029. The mutation was checked by PCR using primers oANG1/oAB114.

AG202
PY79 *yutG::erm*
PY79 *yutG::erm* mutant strain was constructed by transforming a Gibson assembled *yutG::erm* fragment into WT PY79 (BDR11/AG101). For generation of the Gibson assembled fragment a ca. 1.8 kb upstream *yutG* fragment was amplified using primers oIR1113/oANG17 and a ca. 1.8kb downstream *yutG* fragment using primers oANG18/oIR1116. The *loxP* site flanked *erm* marker was amplified using plasmid pWX467 from strain cWX865 (AG110) as templet and primers oJM028/oJM29. The mutation was checked by PCR using primers oANG2/oAB114.

AG203
PY79 *pgpB::kan*
PY79 *pgpB::kan* mutant strain was constructed by transforming a Gibson assembled *pgpB::kan* fragment into WT PY79 (BDR11/AG101). For generation of the Gibson assembled fragment a ca. 1.8 kb upstream *pgpB* fragment was amplified using primers oIR1117/oANG19 and a ca. 1.8kb downstream *pgpB* fragment using primers oANG20/oIR1120. The *loxP* site flanked *kan* marker was amplified using plasmid pWX470 from strain cWX860 (AG109) as templet and primers oJM028/oJM29. The mutation was checked by PCR using primers oANG3/oAB114.

AG208
PY79 *ypjQ::tet, yutG::erm*
gDNA from strain AG202 (PY79 *yutG::erm*) was used to transform strain AG201 (PY79 *ypjQ::tet*) to yield strain PY79 *ypjQ::tet yutG::erm*. The strain was confirmed by PCR using primers oAB114/oANG1 (for *ypjQ::tet* mutation) and oAB114/oANG2 for the *yutG::erm* mutation.

AG209
PY79 *ypjQ::tet, yutG::erm, pgpB::kan*
gDNAs from strains AG202 (PY79 *yutG::erm*) and AG203 (PY79 *pgpB::kan*) were transformed together into strain AG201 (PY79 *ypjQ::tet*) to yield strain PY79 *ypjQ::tet yutG::erm pgpB::kan*. The strain was confirmed by PCR using primers oAB114/oANG1 for the *ypjQ::tet* mutation, oAB114/oANG2 for the *yutG::erm* mutation and oAB114/oANG3 for the *pgp::kan* mutation.

AG216
PY79 *ypjQ::lox72 yutG::lox72*
To construct this strain, the antibiotic markers from strain AG208 (PY79 *ypjQ::tet, yutG::erm*) were removed using plasmid pDR244 (pCre-spec plasmid) (AG113). The removal of the antibiotic markers was checked by PCR using primers oANG1/oIR1112 (*ypjQ*) and oANG2/oIR1116 (*yutG*).

PY79 *ypjQ::lox72 yutG::lox72 pgpB::lox72*
To construct this strain the antibiotic markers from strain AG209 (PY79 *ypjQ::tet yutG::erm pgpB::kan*) were removed using plasmid pDR244 (pCre-spec plasmid) (AG113). The removal of the antibiotic markers was checked by PCR using primers oANG1/oIR1112 (*ypjQ*) and oANG2/oIR1116 (*yutG*) and oANG3/oIR1120 (*pgpB*).

AG228
PY79 *pgpB::lox72*
To construct this strain the antibiotic markers from strain AG203 (PY79 *pgpB::kan*) was removed using plasmid pWX493 (pCre-erm plasmid) (AG114). The removal of the antibiotic marker was checked by PCR using primers oANG3/oIR1120 (*pgpB*).

AG233
PY79 *ypjQ::lox72 yutG::lox72 pgpB::lox72 bcrC::erm*
Strain AG217 (PY79 *ypjQ::lox72 yutG::lox72 pgpB::lox72*) was transformed with gDNA from strain BIR636 (AG121). The strain was confirmed by PCR using primers oYY30/oYY31 (*bcrC::erm*), oANG1/oIR1112 (*ypjQ*), oANG2/oIR1116 (*yutG*) and oANG3/oIR1120 (*pgpB*).

AG240

PY79 *ypjQ::lox72 yutG::lox72 pgpB::lox72 bcrC::lox72*
To construct this strain the antibiotic marker from strain AG233 (PY79 *ypjQ::lox72 yutG::lox72 pgpB::lox72 bcrC::erm*) was removed using plasmid pDR244 (pCre-Spec plasmid) (AG113). The removal of the antibiotic marker was checked by PCR using primers oYY30/oYY31 (*bcrC*) and the gene deletion was checked with primers oANG1/oIR1112 (*ypjQ*), oANG2/oIR1116 (*yutG*) and oANG3/oIR1120 (*pgpB*).

AG289
PY79 *bcrC::lox72*
To construct this strain the antibiotic marker from strain AG290 (PY79 *bcrC::erm*) was removed using plasmid pDR244 (pCre-spec plasmid) (AG113). The removal of the antibiotic markers was checked by PCR using primers oYY30/oYY31 (*bcrC*).

AG290
PY79 *bcrC::erm*
To construct this strain, the *bcrC::erm* marker from strain BIR636 (AG121) was back crossed into WT PY79 (AG101). The *bcrC::erm* mutation was checked by PCR using primers oYY30/oYY31 (*bcrC*).

AG303
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec*, *ycgO::cat* **(PY79 *i-pgpP ycgO::cat*)**
gDNA from *B. subtilis* strain BKM400 (AG160) containing the *ycgO::cat* marker was introduced into the IPTG-inducible *pgpP* strain PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec* (PY79 *i-pgpP*) (AG177). Transformants were selected on Cam5 plates and in the presence of 250 - 500 µM IPTG.

AG321
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpA$_{EC}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpA$_{EC}$***)
Plasmid pCB095-*pgpA$_{EC}$* from *E. coli* strain AG315 allowing for xylose inducible *pgpA$_{EC}$* expression was linearized with ScaI-HF and introduced into the IPTG inducible *pgpP B. subtilis* strain AG303. Transformants were selected on MLS plates and in the presence of 250 - 500 µM IPTG.

AG323
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpC$_{EC}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpC$_{EC}$***)
Plasmid pCB095-*pgpC$_{EC}$* from *E. coli* strain AG316 allowing for xylose inducible *pgpC$_{EC}$* expression was linearized with ScaI-HF and introduced into the IPTG inducible *pgpP B. subtilis* strain AG303. Transformants were selected on MLS plates and in the presence of 250 - 500 µM IPTG.

AG325
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpP$_{SA}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpP$_{SA}$***)
Plasmid pCB095-*pgpP$_{SA}$* from *E. coli* strain AG317 allowing for xylose inducible *pgpP$_{SA}$* expression was linearized with ScaI-HF and introduced into the IPTG inducible *pgpP B. subtilis* strain AG303. Transformants were selected on MLS plates and in the presence of 250 - 500 µM IPTG.

AG327
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpP$_{BS}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpP$_{BS}$***)
Plasmid pCB095-*pgpP$_{BS}$* from *E. coli* strain AG318 allowing for xylose inducible *pgpP$_{BS}$* expression was linearized with ScaI-HF and introduced into the IPTG inducible *pgpP B. subtilis* strain AG303. Transformants were selected on MLS plates and in the presence of 250 - 500 µM IPTG.

AG329
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::PxylA-pgpP$_{BS}$* (*erm*) (**PY79 *i-pgpP Pxyl-pgpP-His***)
Plasmid pCB095-*pgpP-His* from *E. coli* strain AG319 allowing for xylose inducible *pgpP-His* expression was linearized with ScaI-HF and introduced into the IPTG inducible *pgpP B. subtilis* strain AG303. Transformants were selected on MLS plates and in the presence of 250 - 500 µM IPTG.

AG343
PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan)* Δ*pgpP::spec ycgO::Pxyl (erm)* (**PY79 *i-pgpP* (EV)**)

The empty plasmid pCB095 from *E. coli* strain AG143/CDR2113 and containing the xylose inducible promoter without an insert was linearized with ScaI-HF and introduced into the IPTG inducible *pgpP B. subtilis* strain AG303 to yield an empty vector (EV) control strain. Transformants were selected on MLS plates and in the presence of 250 - 500 µM IPTG.

AG345

PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan) ΔpgpP::spec ycgO::PxylA-pgpP$_{D34N}$-His* (*erm*) (**PY79 *i-pgpP PxylA-pgpP$_{D34N}$-His***)

Plasmid pCB095-*pgpP$_{D34N}$-His* from *E. coli* strain AG341 allowing for xylose inducible *pgpP$_{D34N}$-His* expression was linearized with ScaI-HF and introduced into the IPTG inducible *pgpP B. subtilis* strain AG303. Transformants were selected on MLS plates and in the presence of 250 - 500 µM IPTG.

AG347

PY79 *yhdG::Pspank-pgpP$_{BS}$ (kan) ΔpgpP::spec ycgO::PxylA-pgpP$_{D36N}$-His* (*erm*) (**PY79 *i-pgpP PxylA-pgpP$_{D36N}$-His***)

Plasmid pCB095-*pgpP$_{D36N}$-His* from *E. coli* strain AG342 allowing for xylose inducible *pgpP$_{D36N}$-His* expression was linearized with ScaI-HF and introduced into the IPTG inducible *pgpP B. subtilis* strain AG303.

BIR1527

PY79 *ycgO::Pspank-pgsA(spec)*

This strain was constructed by transforming strain PY79 with ScaI linearized pIR469 [*ycgO::Pspank-pgsA(specR)*] plasmid. In this manner the *Pspank::pgsA$_{BS}$ (spec)* fragment for IPTG inducible *pgsA* expression was inserted into the *B. subtilis ycgO* gene. This strain still contains a WT copy of the *pgsA* gene at the native locus.

BIR1532

PY79 *ycgO::Pspank-pgsA(spec) ΔpgsA::erm* (PY79 *i-pgsA*)

This is a *B. subtilis* strain for IPTG-inducible *pgsA* expression from the spank promoter. The *B. subtilis* strain PY79 *ycgO::Pspank-pgsA(spec)* (strain BIR1527) was transformed with a Gibson assembled *pgsA::erm* fragment to replace the *pgsA* gene at the native locus with an erm marker. For generation of the Gibson assembled fragment a *loxP*-site flaked *erm* marker was amplified with primers oJM028/oJM029 from plasmid pWX467 (strain AG110) and assembled with an approximately 1.5 kb upstream *pgsA* fragment generated by PCR using primers oIR1383/oIR1384 and an approximately 1.5 kb downstream fragment generated by PCR using primers oIR1385/oIR1386 and using PY79 gDNA as template. The three fragments were assembled in a Gibson reaction and transform into strain BIR1527. Transformants were selected on MLS plates in the presence of 250 - 500 µM IPTG.

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