SUPPLEMENTAL INFORMATION

The phosphatidic-acid pathway enzyme PlsX plays both catalytic and channeling roles in bacterial phospholipid synthesis

Diego E. Sastre[1,5], André A. Pulschen[1], Luis G. M. Basso[2], Jhonathan S. Benites Pariente[1], Caterina G. C. Marques Netto[3], Federico Machinandiarena[4], Daniela Albanesi[4], Marcos V.A.S. Navarro[5], Diego de Mendoza[4 *], Frederico J. Gueiros-Filho[1*]

[1]Instituto de Química, Departamento de Bioquímica, Universidade de São Paulo, São Paulo, Brazil.

[2] Departamento de Física, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil

[3]Departamento de Química, Universidade Federal de São Carlos (UFSCar), São Carlos, Brazil.

[4]Instituto de Biología Molecular y Celular de Rosario (IBR), and Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina.

[5]Instituto de Física de São Carlos, Departamento de Biofísica Molecular, Universidade de São Paulo, São Carlos, Brazil.

Running title: *Catalytic and channeling roles of PlsX*

***To whom correspondence should be addressed**: Frederico J. Gueiros-Filho, Instituto de Química, IQ- USP, Av. Prof. Lineu Prestes 748, CEP05508000, São Paulo, SP, Brazil. Phone: (+55-11-3091-9101), E- mail: fgueiros@iq.usp.br; Diego de Mendoza**,** Instituto de Biologia Molecular y Celular de Rosario, IBR- Conicet, Esmeralda and Ocampo, S2002LRK Rosario, Santa Fe, Argentina. Phone: (+54-341-4237070. Ext: 621). E-mail: demendoza@ibr-conicet.gov.ar

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SUPPLEMENTARY TABLES

Table S1

Table S1. List of strains and plasmids used in this study

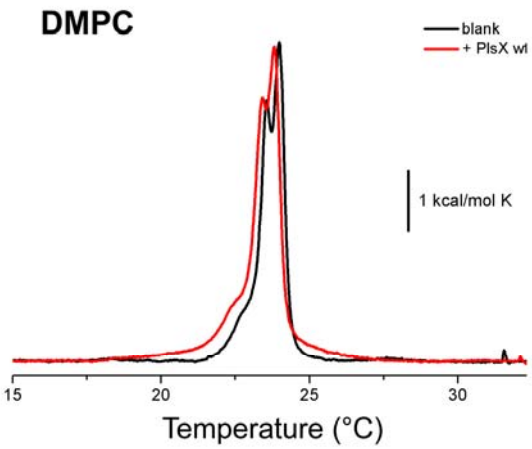
Strains/ Plasmids	Relevant features or genotype	Construction, source or reference
B. subtilis		
PY79	Prototroph SPβ	Laboratory stock
DS09	PY79 *amy*E::cat (P*xyl*-*gfp*-plsX)	pDS1 integration into strain PY79
DS28	PY79 (cat Pxyl-*pls*X), (erm P*spac*-fabD)	derivated from LP39 (1)
DS69	PY79 (cat Pxyl-*pls*X), (erm P*spac*-fabD); *amy*E::spc (P*hy*-*spank*-*gfp*-*pls*X)	pDS2 integration into strain DS28 (2)
DS34	DS28 *amy*E::spc (P*hy*-*spank*-*gfp*-*pls*X AH2(+2)	This study
DS48	DS28 *amy*E::spc (P*hy*-*spank*-*gfp*-*pls*X AH (+3))	This study
DS58	DS28 *amy*E::spc (P*hy*-*spank*-*gfp*-*pls*X L235E)	This study
DS160	DS28 *amy*E::spc (P*hy*-*spank*-*gfp*-*pls*X L254E)	This study
DS161	DS28 *amy*E::spc (P*hy*-*spank*-*gfp*-*pls*X K271A)	This study
DS170	DS28 *amy*E::spc (P*hy*-*spank*-*gfp*-*pls*X-hbsu)	This study
DS171	DS28 *amy*E::spc (P*hy*-*spank*-*gfp*-*pls*X L254E-*hbsu*)	This study
E. coli		
DH5α	F-, ϕ80lacZΔM15, Δ (lacZYAargF) U196, recA1, endA1, hsdR17, (rK-, mK+), phoA, supE44, λ-, thi-1, gyrA96, relA1	Laboratory stock
BL21 (DE3)	F⁻ *ompT gal dcm lon hsdS$_B$*($r_B^- m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K\text{-}12}$(λ^S)	Laboratory stock
DS52	*E.coli* BL21 pET-24b-*pls*X *wt*	This study
DS85	*E.coli* BL21 pET-24b-*pls*X AH2(+2)	This study
DS87	*E.coli* BL21 pET-24b-*pls*X AH2(+3)	This study
DS88	*E.coli* BL21 pET-24b-*pls*X L235E	This study
DS125	*E.coli* BL21 pET-24b-*pls*X L254E	This study
DS126	*E.coli* BL21 pET-24b-*pls*X Y276E	This study
DS130	*E.coli* BL21 pET-24b-*pls*X V262E	This study
DS131	*E.coli* BL21 pET-24b-*pls*X K271A	This study
DS139	*E.coli* BL21 pET-24b-*pls*X L258E-A259E	This study
DS140	*E.coli* BL21 pET-24b-*pls*X K264A	This study
DS128	*E.coli* BL21 pREP4 pQE30-*tev*-*acp*	Laboratory stock
Plssmids		
pDR111	*bla amy*E3′*spc* P*hy*-*spank* lacI *amy*E5′	Laboratory stock
pDS2	*bla amy*E3′*spc* P*hy*-*spank*-*gfp*-*pls*X *amy*E5′	derivated of pDR111 (2)
pET24b(+)	5.3-kb plasmid allowing C-terminal protein fusion to 6X-His; Kan^r	Novagen
pDS2_1	*bla amy*E3′*spc* P*hy*-*spank*-*gfp*-*pls*X AH2(+2) *amy*E5′	This study
pDS2_2	*bla amy*E3′*spc* P*hy*-*spank*-*gfp*-*pls*X AH (+3)*amy*E5′	This study
pDS2_3	*bla amy*E3′*spc* P*hy*-*spank*-*gfp*-*pls*X L235E *amy*E5′	This study
pDS2_4	*bla amy*E3′*spc* P*hy*-*spank*-*gfp*-*pls*X L254E *amy*E5′	This study
pDS2_5	*bla amy*E3′*spc* P*hy*-*spank*-*gfp*-*pls*X K271A *amy*E5′	This study
pDS2_6	*bla amy*E3′*spc* P*hy*-*spank*-*gfp*-*pls*X-*hbsu amy*E5′	This study
pDS2_7	*bla amy*E3′*spc* P*hy*-*spank*-*gfp*-*pls*X L254E-*hbsu amy*E5′	This study
pDS_a	pET-24b-*pls*X *wt*	This study
pDS_b	pET-24b-*pls*X AH2(+2)	This study
pDS_c	pET-24b-*pls*X AH2(+3)	This study
pDS_d	pET-24b-*pls*X L235E	This study
pDS_e	pET-24b-*pls*X L254E	This study
pDS_f	pET-24b-*pls*X Y276E	This study
pDS_g	pET-24b-*pls*X V262E	This study
pDS_h	pET-24b-*pls*X K271A	This study
pDS_i	pET-24b-*pls*X L258E-A259E	This study
pDS_j	pET-24b-*pls*X K264A	This study
pREP4	pQE30-*tev*-*acp*	Laboratory stock

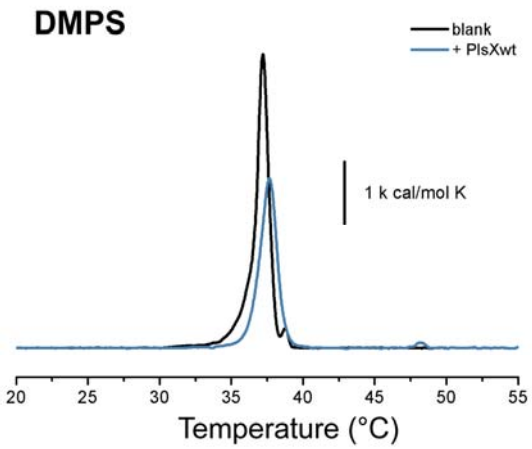
Table S2

Table S2. List of oligonucleotide primers used in this study

Primer name	sequence (5´-3´)
NheI-rbs-GFP F	CCACGCTAGCAAAGGTGGTGAATCTGGCATGAGTAAAGGAGAAGAAC
SphI-PlsX stop R	CGCCGCATGCCTACTCATCTGTTTTTTCTTC
SphI-HBsu stop R	CCTATGCATGCTTATTTTCCGGCAACTGCGTC
HBsu-linker-PlsX R	GTTCATGCGTCCGACTGCTTCCTCCTCATCTGTTTTTTCTTCTTTCAC
PlsX-linker-HBsu F	GAGGAGGAAGCAGTCGGACGCATGAACAAAACAGAACTTATCAATGCGG
NdeI-PlsX F	GCACATATGAGAATAGCTGTAGATG
XhoI-stop PlsX R	GTAGGATCCCTCGAGGTACTCATCTGTTTTTTC
PlsX_AH2(+2) F	CTCTGCGTTGTCAATTGCGGAGTTTAAAATGATGAGAG
PlsX_AH2(+2) R	CTCTCATCATTTTAAACTCCGCAATTGACAACGCAGAG
PlsX_AH2(+3) F	GCTCTGCGTTGTCAATTGAGGAGCTCTTTAAAATGATGAGAG
PlsX_AH2(+3) R	CTCTCATCATTTTAAAGAGCTCCTCAATTGACAACGCAGAGC
PlsX_L235E F	GTTACACTCAAAACGGAGGAAGGCTCTGCGTTG
PlsX_L235E R	CAACGCAGAGCCTTCCTCCGTTTTGAGTGTAAC
PlsX_L254E F	CGTAATGACGTCTACTGAGACATCCAAGCTTGCAG
PlsX_L254E R	CTGCAAGCTTGGATGTCTCAGTAGACGTCATTACG
PlsX_L258E-A259E F	GTCTACTTTGACATCCAAGGAGGAAGCAGCTGTGCTGAAAC
PlsX_L258E-A259E R	GTTTCAGCACAGCTGCTTCCTCCTTGGATGTCAAAGTAGAC
PlsX_V262E F	CAAGCTTGCAGCAGCTGAGCTGAAACCAAAATTG
PlsX_V262E R	CAATTTTGGTTTCAGCTCAGCTGCTGCAAGCTTG
PlsX_K264A F	GCAGCAGCTGTGCTGGCACCAAAATTGAAAG
PlsX_K264A R	CTTTCAATTTTGGTGCCAGCACAGCTGCTGC
PlsX_K271A F	CAAAATTGAAAGAAATGGCAATGAAAATGGAGTATTC
PlsX_K271A R	GAATACTCCATTTTCATTGCCATTTCTTTCAATTTTG
PlsX_Y276E F	TGAAAATGGAGGATTCGAATTATGGCG
PlsX_Y276E R	CGCCATAATTCGAATCCTCCATTTTCA

SUPPLEMENTARY FIGURES





	L/P	Tm (°C)	ΔH (kcal/mol)	ΔT ½ (°C)
blank		24.0	5.1	0.8
+PlsX wt	136	23.8	6.7	0.9

	L/P	Tm (°C)	ΔH (kcal/mol)	ΔT ½ (°C)
blank		37.2	6.1	0.9
+PlsX wt	136	37.6	4.3	1.4

Figure S1. Interaction of PlsX with DMPC and DMPS LUVs. Effect of PlsX wt on the thermotropic behavior of lipid bilayer membranes probed by DSC. Excess heat capacity of DMPC (left panel) and DMPS (right panel) in the absence (black) and in the presence of PlsX at lipid-to-protein (L/P) molar ratio of 136:1. Lipid concentration was 800 μM in all cases. The phase transition temperature, T_m, the calorimetric enthalpy change, ΔH, and the linewidth at half height, $\Delta T_{1/2}$, were obtained from analysis of the thermograms with MicroCal Origin software. Data shown are representative of two independent experiments.

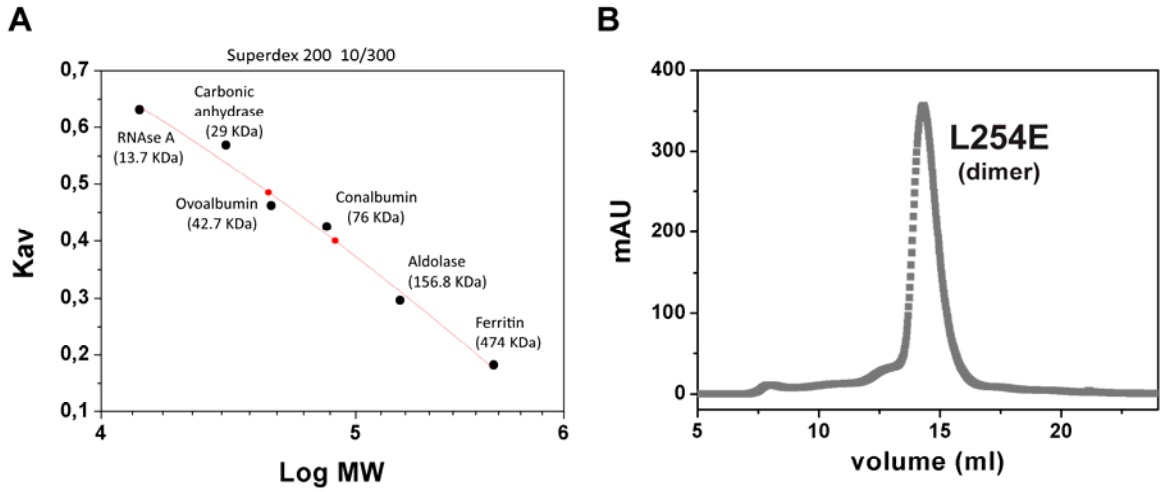


Figure S2. Calibration of Superdex S-200 10/300 and gel filtration of PlsX^{L254E} mutant. *A.* The red points are indicating the peaks of PlsX dimer (Kav=0.398) and PlsX monomer (Kav=0.485). *B.* Purified PlsX L254E mutant was a soluble dimeric protein in a S-200 gel filtration chromatography. Data shown are representative of two independent experiments.

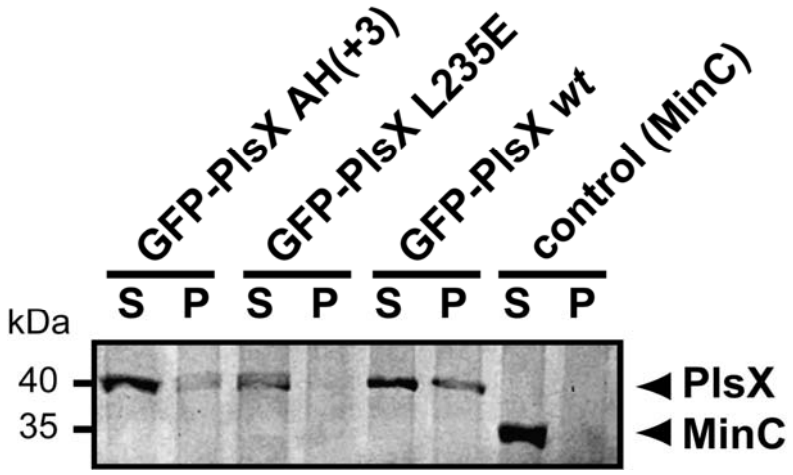


Figure S3. Cosedimentation assay showing the interaction of PlsX *wt* with liposomes. No interaction can be seen in the case of L235E mutant and MinC (negative control). S: supernatant of ultracentrifugation. P: pellet of ultracentrifugation. L: ladder. Data shown are representative of two independent experiments.

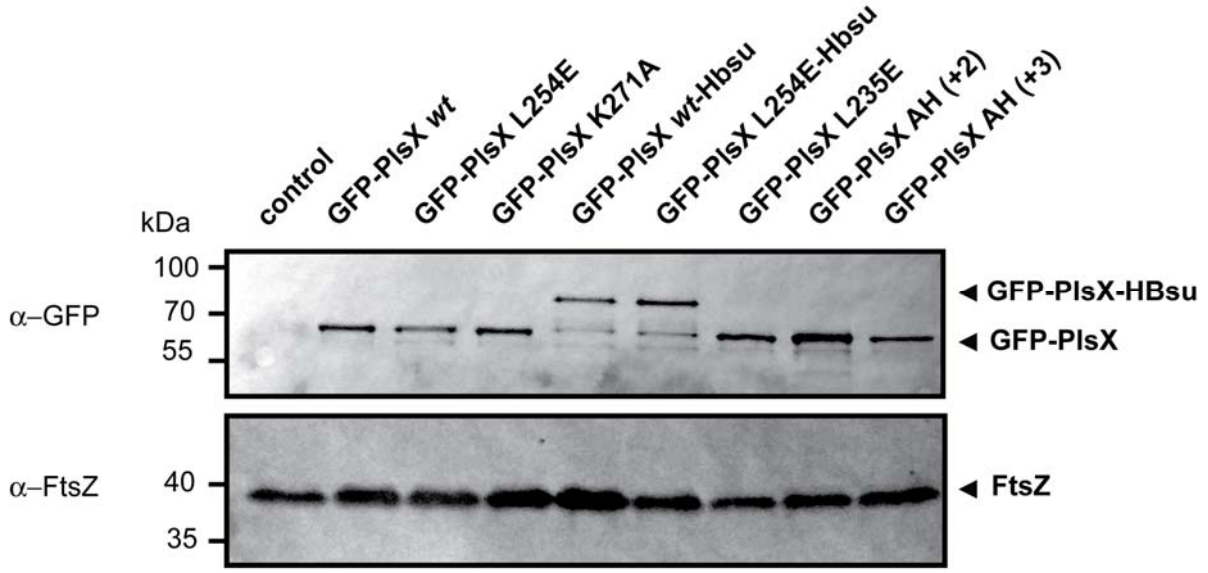


Figure S4. Western blotting assay. Cell lysates of *B. subtilis* DS28 (control) containing different relevant mutations of GFP-PlsX fusions were subjected to 10% SDS-polyacrylamide gel electrophoresis. GFP- PlsX was detected using the polyclonal rabbit anti-GFP antibody (upper panel). The cellular amount of FtsZ, used as a normalization control, was analyzed with anti-FtsZ antibody (lower panel). Data shown are representative of two independent experiments.

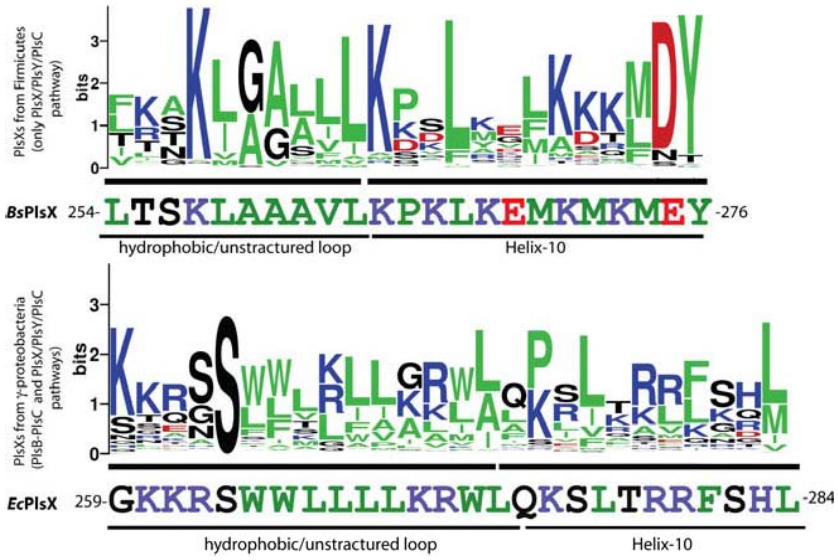


Figure S5. Sequence logo of aminoacid residues of hydrophobic loop and helix-10 from different PlsXs proteins. 150 sequences from Firmicutes and 150 from γ-proteobacteria were obtained from NCBI database (www.ncbi.nlm.nih.gov/protein). Multiple sequence alignment was performed using T-Coffee (3) and the Logo was obtained using WebLogo online server (4).

References

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