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Deciphering the recombinant thermostable phosphatidylcholine-specific phospholipase C activity from Bacillus thuringiensis: biochemical and interfacial properties

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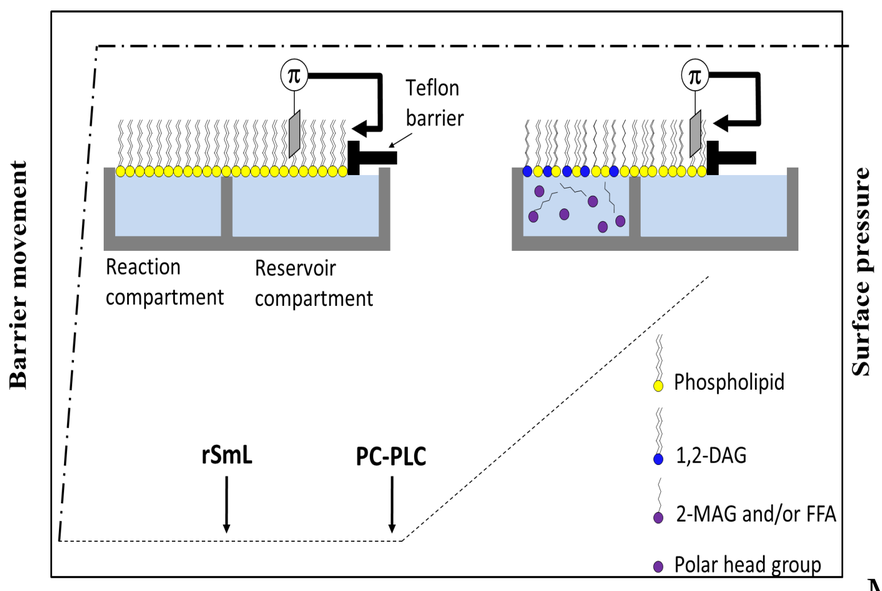
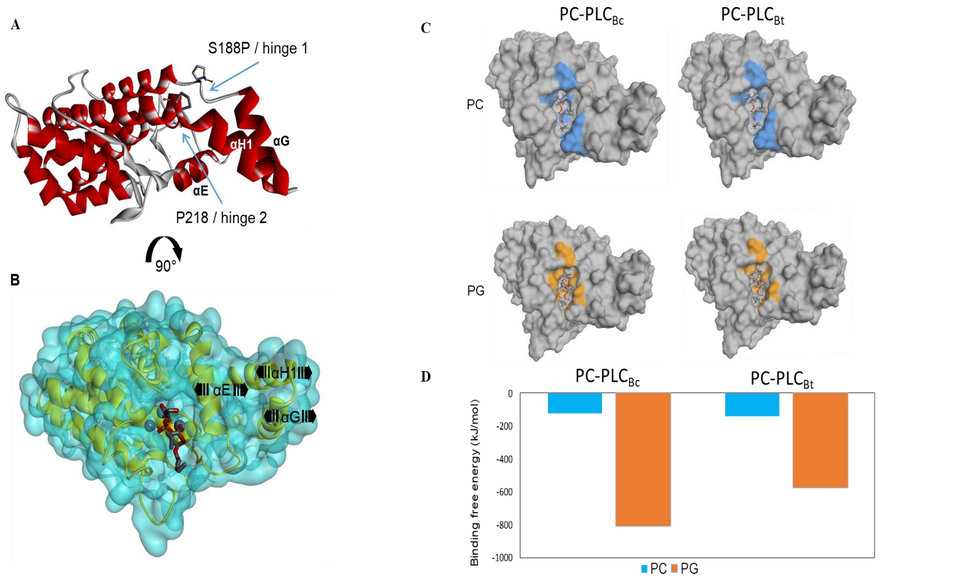
❖**Abstract**

A novel alkaline thermostable phosphatidylcholine-specific phospholipase C (PC-PLC_{Bt}) was expressed in *E. coli* system. Recombinant PC-PLC_{Bt} (rPC-PLC_{Bt}) activity and thermostability were shown to be significantly dependent on the Zn^{2+}. The maximum rPC-PLC_{Bt} catalytic activity was found to be 1372 $U.mg^{-1}$ in the presence of 0.1 mM Zn^{2+} and at 60 °C using an Egg PC as substrate. The interfacial kinetic data show that nPC-PLC_{Bt} and rPC-PLC_{Bt} display similar substrate specificity on various phospholipid monolayers. The maximal rPC-PLC_{Bt} activities were recorded, at decreasing order, on 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE), 1,2-diacyl-*sn*-phosphoglycerol (DLPG), and 1,2-diacyl-*sn*-phosphoserine (PS) monolayers at interfacial surface pressures of 15, 25, 20, and 25 $mN.m^{-1}$, respectively. The highest activities were recorded on the PC monolayer and shown to be 121.61 and 40.13 $mmol.cm^{-2}.min^{-1}.M^{-1}$ for native and recombinant PC-PLC_{Bt}, respectively. Interestingly, compared to all known *Bacillus* PLCs, both PC-PLC_{Bt} forms showed an exclusive capacity to hydrolyze the DLPG film with a more pronounced rate of hydrolysis for the native form with a specific activity of 58.29 $mmol.cm^{-2}.min^{-1}.M^{-1}$. Therefore, the high enzyme level production of about 14 $mg.\ L^{-1}$, the thermostability as well as the broad phospholipid specificity of PC-PLC_{Bt} represents a great potential in the crude oil refining industry.

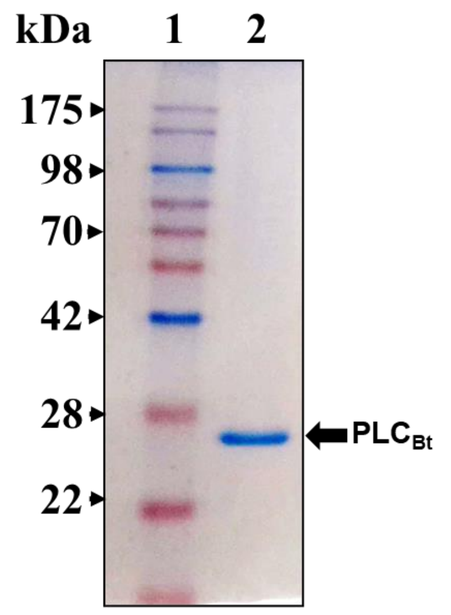
❖**Keywords:** *B. thuringiensis* IL14-PC-PLC; higher thermostability and substrate specificity; interfacial and cytotoxic properties.



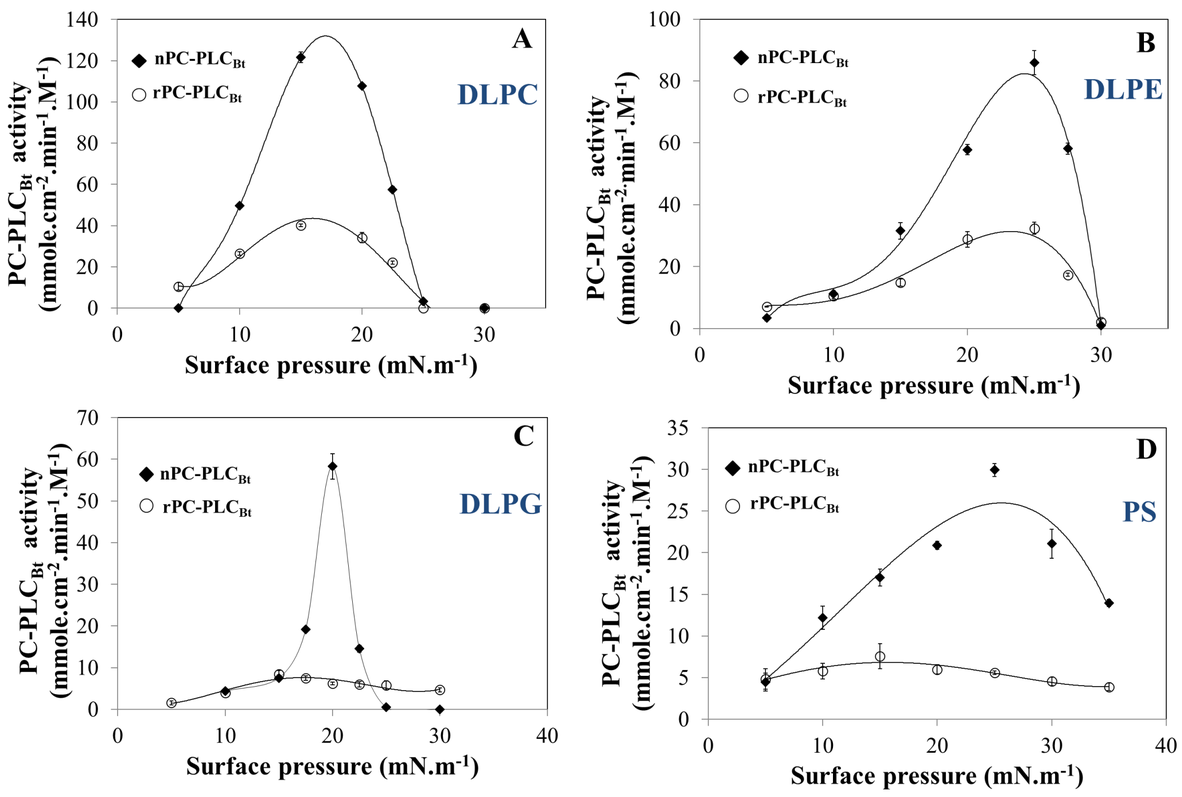
Principle of **the coupled assay** for determining PC-PLC_{Bt} activity using phospholipid monolayers as substrates. rSmL was injected first into the subphase at a fixed final amount of 40 ng and nPC-PLC_{Bt} or rPC-PLC_{Bt} was added 10 min later.



Molecular modeling, docking experiment and MM/PBSA simulations. **(A)** Ribbon representation of PC-PLC_{Bt} molecular model. P188 and P218 are depicted as sticks **(B)** Docking of a PG into the PC-PLC_{Bt} model. The enzyme backbone is depicted as a ribbon covered by a semitransparent Van der Walls surface, while PG is depicted as sticks and zinc ion as a grey sphere. The hatched arrows materialize the potential motions of the protein domains**. (C)** Docking of a PC and of a PG into the PC-PLC_{Bc} x-ray-structure or into the PC-PLC_{Bt} model. Residues that show hot-spot interactions with PC during the simulations are colored in blue while ones interacting with PG are in orange **(D)** MM/PBSA binding free energy calculations for PC-PLC_{Bc} and PC-PLC_{Bt} with PC or PG ligands.



SDS-PAGE (15% of acrylamide) analysis of rPC-PLC_{Bt} at final purification step (Sephadex G-75 column). The gel was stained with Coomassie blue to reveal the proteins. **Lane 1**: molecular mass marker, **lane 2**: Purified enzyme fraction eluted from G-75 column.



Variation with a surface pressure of nPC-PLC_{Bt} and rPC-PLC_{Bt} on various phospholipids; DLPC **(A)**, DLPE **(B),** DLPG**(C),** and PS **(D).** PLC activity is expressed as the number of moles of substrates hydrolyzed by unit time and the unit surface of the reaction compartment of the "zero-order" trough.

Conclusions

The expression of the PC-PLC_{Bt} in the *E. coli* system leads to a soluble and fully active enzyme with a specific activity of around 1372 $U.mg^{-1}$ measured at 60 °C on a PC emulsion in the presence of 0.1 mM Zn^{2+}. Purified rPC-PLC_{Bt} kept at almost, its biochemical properties with an improvement of the Zn^{2+} role required for catalysis and maintaining the integrity of the structure of rPC-PLC_{Bt}. The thermoactivity, the thermostability, and the high amount of active soluble protein indicate the great potential of this PLC for biotechnological applications, especially for oil degumming carried out under extreme conditions. Nevertheless, in addition to these biochemical criteria, substrate specificity and kinetic properties remain the most determinants factors for a suitable industrial application. In that context, a comparative kinetic study was investigated with various phospholipids. Findings proved that both native and recombinant PC-PLC_{Bt} forms can hydrolyze phospholipid monolayers with different efficiency and various optimal surface pressures ranging from 15 to 25 $mN.m^{-1}$. The PC is the major hydrolyzed substrate followed by the PE however the hydrolysis rate is low with PG and PS. Interfacial binding to phospholipid monolayers at high surface pressure can be correlated to the prominent direct hemolytic power and cytotoxic potential of this new PLC on tumoral mammalians cells. Such highly potent rPC-PLCBt activity could be exploited for therapeutic and pharmacological purposes as a bactericidal and anti-tumor agent.