Biochemical characterization of phospholipases D and development of assays to measure their activities.

Phospholipases D (PLD) are key enzymes involved in numerous processes in all living organisms. PLD catalyze, notably, the hydrolysis of different phospholipids (PL) generating phosphatidic acid (PA). PA is an important moiety at the crossroads of multiple metabolic pathways and it is involved in signaling reactions, cancer genesis in mammals, bacterial infections and reproduction in plants.

Most PLD assays developed so far were either discontinuous or based on the indirect determination of choline released during PLD-catalyzed phosphatidylcholine (PC) hydrolysis, making kinetic characterization difficult. Therefore we developed a direct, specific, and continuous PLD assay that is based on the chelation enhanced fluorescence property of 8-hydroxyquinoline (8HQ) following Ca2+ complexation with PLD-generated PA.

This test allowed us to characterize newly identified PLD in the bacterium *Dechloromonas aromatica RCB* and in plants. Compared to already known PLDs, this bacterial PLD has a strong preference for phosphatidylethanolamine (PE) over all other PL, especially PC and has a typoselectivity for unsaturated PE that does not exist for PC. Interestingly, the recombinant expression of this new bacterial PLD led to a stunning change in PL composition and amount in *E. coli*, especially for PA.

These findings offer new perspectives on PA production and regulation in bacteria and plants.