Cloning a Phospholipase A₂ from Hemocytes of Spodoptera exigua Infected with Xenorhabdus nematophila

Sunghwan Cho, Sangjin Kang and Yonggyun Kim

Department of Agricultural Biology, Andong National University, Andong, Korea

Phospholipase A₂ (PLA₂) is an enzyme catalyzing phospholipidsat sn-2 position to produce a free fatty acid, mainly arachidonic acid. PLA₂ is implicated to express cellular immunity in insects. An entomopathogenic bacterium, *Xenorhabdus nematophila*, inhibits PLA₂ to induce host immunosuppression. A preliminary work using various PLA₂ antibodies indicates that hemocytes of *Spodoptera exigua* express an inducible PLA₂ in response to bacterial infection. The inducible PLA₂ reacted to secretory type of PLA₂ antibody. Based on known PLA₂ sequence of *Drosophila melanogaster*, we designed type IIIa-secretory PLA₂ degenerate primers. An RT-PCR (3'-RACE) 600 bp product and exhibited high homology with known PLA₂ sequence. Following 5'-RACE resulted in a complete cDNA sequence of 1151 bp size. This sequence contains amidation site, casein kinase II phosphorylation site, cAMP- and cGMP-dependent protein kinase phosphorylation site, N-glycosylation site, N-myristorylation site, protein kinase C phosphorylation site, and PLA₂ histidine active site. RT-PCRs showed that the hemocyte PLA₂ is specificity expressed in hemocytes and also that the PLA₂ is expressed in response to bacterial infection.