

Biochemical Characterization of a Phospholipase A₂ from *Photobacterium damsela* subsp. *piscicida*

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Photobacterium damsela subsp. *piscicida* (*Phdp*) is the causative agent of fish photobacteriosis (pasteurellosis) in cultured cobia (*Rachycentron canadum*) in Taiwan. A component was purified from the extracellular products (ECP) of the bacterium strain 9205 by fast protein liquid chromatography (FPLC) and identified as a phospholipase. An N-terminal sequence of 10 amino acid residues, QDQPNLDPGK, was determined by mass spectroscopy (MS) and found to be identical with that of another *Phdp* phospholipase (GenBank accession no. BAB85814) at positions 21 to 30. The corresponding gene sequence of the phospholipase (GenBank accession no. AB071137) was employed to design primers for amplification of the sequence by the polymerase chain reaction (PCR). The PCR products were transformed into *Escherichia coli*, and a recombinant protein product was obtained which was purified as a His-tag fusion protein by Ni-metal affinity chromatography. A single 43-kDa band was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Phosphatidylcholine was degraded by this protein to lysophosphatidylcholine and a fatty acid. These products were characterized by thin-layer (TLC) and gas chromatography (GC), respectively, allowing the identification of the protein as a phospholipase A₂. The recombinant protein had maximum enzymatic activity between pH 4 and 7, and at 40 °C. The activity was inhibited by Zn²⁺ and Cu²⁺, activated by Ca²⁺ and Mg²⁺, and completely inactivated by dexamethasone and *p*-bromophenacyl bromide. A rabbit antiserum against the recombinant protein neutralized the phospholipase A₂ activity in the ECP of *Phdp* strain 9205 and the recombinant protein itself. The recombinant protein was toxic to cobia of about 5 g weight with an LD₅₀ value between 2 and 4 μg protein/g fish. The results revealed phospholipase A₂ as a fish toxin in the ECP of *Phdp* strain 9205.

Key words: *Photobacterium damsela* subsp. *piscicida*, *Rachycentron canadum*, Phospholipase A₂