Purification of a Toxic Metalloprotease Produced by the Pathogenic *Photobacterium damselae* subsp. *piscicida* Isolated from Cobia (*Rachycentron canadum*)

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The aim of the present study was to purify and characterize a toxic protease secreted by the pathogenic *Photobacterium damselae* subsp. *piscicida* strain CP1 originally isolated from diseased cobia (*Rachycentron canadum*). The toxin isolated by anion exchange chromatography, was a metalloprotease, inhibited by L-cysteine, ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(*b*-aminoethyl ether)*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA), 1,10-phenanthroline, *N*-tosyl-L-phenylalanine-chloromethyl ketone (TPCK), and *N*-Ŭ*p*-tosyl-L-lysine-chloromethyl ketone (TLCK), and showed maximal activity at pH 6.0–8.0 and an apparent molecular mass of about 34.3 kDa. The toxin was also completely inhibited by $HgCl_2$, and partially by sodium dodecyl sulfate (SDS) and $CuCl_2$. The extracellular products and the partially purified protease were lethal to cobia with LD_{50} values of 1.26 and 6.8 μg protein/g body weight, respectively. The addition of EDTA completely inhibited the lethal toxicity of the purified protease, indicating that this metalloprotease was a lethal toxin produced by the bacterium.

Key words: Metalloprotease, *Rachycentron canadum, Photobacterium damselae* subsp. *piscicida*