Cytotoxicity of *Vibrio parahaemolyticus* AHPND toxin on shrimp hemocytes, a newly identified target tissue, involves binding of toxin to aminopeptidase N1 receptor

Waruntorn Luangtrakul[1], Pakpoom Boonchuen[1,2], Phattarunda Jaree[3], Ramya Kumar[4,5], Han-Ching Wang[4,5,*], Kunlaya Somboonwiwat[1,*]

[1] Center of Excellence for Molecular Biology and Genomics of Shrimp, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.
[2] School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Mueang,
Nakhon Ratchasima, Thailand
[3] Center of Applied Shrimp Research and Innovation, Institute of Molecular Biosciences, Mahidol University, Salaya, Nakhon Pathom, Thailand
[4] Department of Biotechnology and Bioindustry Sciences, College of Biosciences and Biotechnology, National Cheng Kung University, Tainan, Taiwan
* Correspondence: kunlaya.s@chula.ac.th (KS); wanghc@mail.ncku.edu.tw (HC);Tel +662-218-5438

Abstract: Acute hepatopancreatic necrosis disease (AHPND) caused by PirAB^{VP}-producing strain of *Vibrio parahaemolyticus*, VP$_{AHPND}$, has seriously impacted the shrimp production. Although the VP$_{AHPND}$ toxin is known as the VP$_{AHPND}$ virulence factor, a receptor that mediates its action has not been identified. An in-house transcriptome of Litopenaeus vannamei hemocytes allows us to identify two proteins from the aminopeptidase N family, *Lv*APN1 and *Lv*APN2, the proteins of which in insect are known to be receptors for Cry toxin. The membrane-bound APN, *Lv*APN1, was characterized to determine if it was a VP$_{AHPND}$ toxin receptor. The increased expression of *Lv*APN1 was found in hemocytes, stomach, and hepatopancreas after the shrimp were challenged with either VP$_{AHPND}$ or the partially purified VP$_{AHPND}$ toxin. *Lv*APN1 knockdown reduced the mortality, histopathological signs of AHPND in the hepatopancreas, and the number of virulent VP$_{AHPND}$ bacteria in the stomach after VP$_{AHPND}$ toxin challenge. In addition, *Lv*APN1 silencing prevented the toxin from causing severe damage to the hemocytes and sustained both the total hemocyte count (THC) and the percentage of living hemocytes. We found that the r*Lv*APN1 directly bound to both rPirA^{VP} and rPirB^{VP} toxins, supporting the notion that silencing of *Lv*APN1 prevented the VP$_{AHPND}$ toxin from passing through the cell membrane of hemocytes. We concluded that the *Lv*APN1 was involved in AHPND pathogenesis and acted as a VP$_{AHPND}$ toxin receptor mediating the toxin penetration into hemocytes. Besides, this was the first report on the toxic effect of VP$_{AHPND}$ toxin on hemocytes other than the known target tissues, hepatopancreas and stomach.

Keywords: Aminopeptidase N1; *Litopenaeus vannamei*; Acute hepatopancreatic necrosis disease (AHPND); *Vibrio parahaemolyticus* causing AHPND (VP$_{AHPND}$); VP$_{AHPND}$ toxin receptor

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