ALF*Pm*3-resistance in AHPND-causing *Vibrio parahaemolyticus* relates to biofilm formation and reduces PirAB^{VP} toxin production

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Abstract: Anti-lipopolysaccharide factor isoform 3 (ALF*Pm*3) from the black tiger shrimp, *Penaeus monodon* exhibits antimicrobial activities against various microorganisms. ALF*Pm*3 can effectively kill the major shrimp pathogen, acute hepatopancreatic necrosis disease (AHPND)-causing *Vibrio parahaemolyticus* (VP$_{AHPND}$) through interfering the bacterial membrane resulting in cell lysis. interfering the bacterial membrane resulting in cell lysis. VP$_{AHPND}$ is a Gram-negative marine bacterium producing the binary PirAB^{VP} toxin, the virulence factor that mediates AHPND and mortality in shrimp. Facing the same challenges as other antibiotic usages, repeated exposure with antimicrobial peptide (AMP) might generates resistant strains of VP$_{AHPND}$ making APHND much more difficult to treat. Thus, this research aims to determine how VP$_{AHPND}$ acquires ALF*Pm*3 resistance. First, VP$_{AHPND}$ was cultured in the medium supplemented with increasing concentration of ALF*Pm*3 to generate the resistant strains. Screening was performed till the minimum inhibitory concentration (MIC) value reached 8 MIC, five clones of ALF*Pm*3-resistant VP$_{AHPND}$ were obtained. The resistant clones showed the enhanced biofilm formation with increasing amount of protein content but not carbohydrates. Surprisingly, we found the decreased level of binary PirAB^{VP} toxin released by all resistant clones suggesting their lower pathogenicity. In conclusions, ALF*Pm*3-resistance in VP$_{AHPND}$ was mediated by enhancing biofilm formation. However, in terms of pathogenicity, this ALF*Pm*3-resistant strains might be less harmful to shrimp because they can no longer produce PirAB^{VP} toxin.

Keywords: Antilipopolysaccharide factor isoform 3; acute hepatopancreatic necrosis disease; *Vibrio parahaemolyticus*; antimicrobial peptide resistance

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