

**A NEW EMS-ASSOCIATED BACTERIUM OF THE GENUS
ROSEATELES FOUND IN SHRIMP**



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ABSTRACT

Early Mortality Syndrome (EMS), which is an unusually high mortality observed within approximately 35 days of rearing post larvae of shrimp in a culture pond, is a serious threat to the shrimp farming industry especially in China, Malaysia, Vietnam and Thailand. Studies have demonstrated that *Vibrio parahaemolyticus* harbouring a plasmid containing toxin genes (VP_{AHPND}) is a major causative agent of EMS in the form of acute hepatopancreatic necrosis disease (AHPND). However, shrimp specimens in a number of shrimp ponds exhibiting EMS did not show the pathological characteristics associated with AHPND and the cause of mortality in those ponds remains unexplained. We hypothesized that there are other pathogens involved in EMS or associated with VP_{AHPND} that cause EMS. In this study, we reported a discovery of a bacterium from the genus *Roseateles* that could kill shrimp in its own right but also work synergistically in mixed challenges to increase the virulence of VP_{AHPND} . In vivo bioassays indicated that the 4-day LC_{50} of *Roseateles* alone for shrimp was 10^5 CFU/ml while that at 10^2 CFU/ml was 45%. However, mortality of shrimp challenged with VP_{AHPND} at 10^3 CFU/ml was 65% on day 3 post immersion challenge, while addition of *Roseateles* at a concentration of 10^2 CFU/ml (i.e., an additional 100 cells) in a co-challenge test with VP_{AHPND} at 10^3 CFU/ml (i.e., 1100 cells total) resulted in 75% cumulative mortality on the same day 3 post challenge. A mixture of both at 10^3 CFU/ml each (2000 cells total) gave 100% mortality in 3 days while 100% mortality from VP_{AHPND} alone required 10^5 CFU/ml (100,000 cells). These results indicate that a combination of *Roseateles* and VP_{AHPND} increases shrimp mortality in a synergistic manner. A specific, nested PCR detection method for shrimp *Roseateles* was developed based on its 16S-23S rRNA intergenic spacer region (ITS region).

KEY WORDS: EARLY MORTALITY SYNDROME (EMS)/ EMS/AHPND
 VP_{AHPND} / *ROSEATELES*.

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