# Mitigation of AHPND based on phenotype switching in *Vibrio parahaemolyticus*

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**ABSTRACT:**
AHPND is a disease caused mainly by *Vibrio parahaemolyticus*. The virulent strains harbor a plasmid encoding for the PirA/B toxin genes. AHPND strains display 2 phenotypic stages. In the planktonic form pirA/B toxins are produced and secreted. In the auto-flocculating form, toxins are not produced, but rather an alkaline phosphatase is produced and secreted. Expression of the toxin genes or the alkaline phosphatase PhoX gene can be used as marker for their phenotypic status. This study aimed at developing AHPND mitigation strategies that profit from the phenotype switching. First a biofloc system was verified, to describe the underlying mechanism behind the AHPND protective effect of a biofloc system in *Litopenaeus vannamei*. First, the results confirmed that a biofloc system maintained at a C/N ratio of 15, improves the water quality and contributes to the nutrition of cultured animals as bioflocs might serve as an additional protein source. Secondly, the study demonstrated that the biofloc system enhances the survival of *L. vannamei* upon challenge with a *V. parahaemolyticus* AHPND strain. Remarkably, the results highlight that in the biofloc system, AHPND-causing *V. parahaemolyticus* possibly switch from virulent planktonic phenotype, producing AHPND toxins, to a non-virulent biofilm phenotype (not producing APHND toxins), as demonstrated by a decreased transcription of flagella-related motility genes (*flaA*, *CheR*, and *fliS*), Pir toxin ($PirB^{VP}$), and AHPND plasmid genes (*ORF14*). In contrast an increased expression of the phenotype switching marker *AlkPhoX* gene was observed in both *in vitro* (in the biofloc) and *in vivo* (in the stomach of biofloc-based shrimp) conditions. Taken together, results suggest that bioflocs steer phenotype switching, contributing to the decreased virulence of *V. parahaemolyticus* AHPND strain towards shrimp postlarvae. In addition, it was found that bamboo powder as a substratum is also inducing a phenotypic switch. This information opens the possibility to combat AHPND not only by trying to eliminate the AHPND-causing *V. parahaemolyticus* from the system but rather to steer the system allowing for a phenotypic switch of *V. parahaemolyticus*