In Depth Investigation into Pathophysiology of the Gut Metagenome and Host Response to *Vp*AHPND In Madagascar–Malaysia Crossbred Black Tiger Shrimp *Penaeus Monodon* Cohort Stocks

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Introduction

The lethal shrimp bacterial infection known as Acute Hepatopancreatic Necrosis Disease (AHPND) is mainly caused by *Vibrio parahaemolyticus* AHPND strain (*Vp*AHPND) bacteria.
The *Vp*AHPND bacteria mainly colonize the stomach and gut regions and release toxins to damage shrimp hepatopancreas (Figures 1&2).
The present study aims to identify the important changes of *Penaeus monodon* gut metagenome and biochemical activities especially in hepatopancreas when infected with *Vp*AHPND (Figure 3).

Materials and Methods

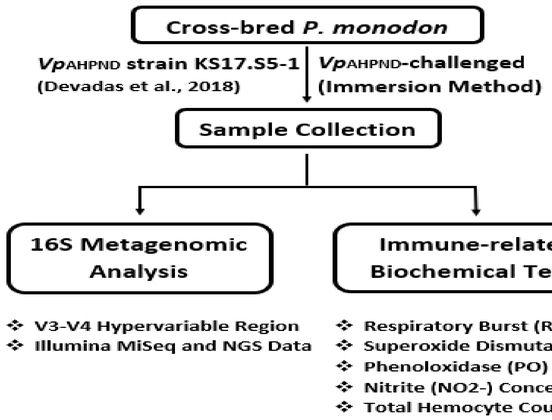


Figure 3. General experimental flow.

- V3-V4 Hypervariable Region
- Illumina MiSeq and NGS Data

- Respiratory Burst (RB) Test (OD 630 nm)
- Superoxide Dismutase (SOD) Activity Test (OD 560 nm)
- Phenoloxidase (PO) Activity Test (OD 490 nm)
- Nitrite (NO_2^-) Concentration Test (OD 540 nm)
- Total Hemocyte Count (THC)

Results and Discussion

- Significant increments or reductions were identified in the *P. monodon* biochemical activities upon *Vp*AHPND infection with respective peaks of (Figure 4):
  - RB- Highest 12 hpi (hours post-infection)
  - SOD activity- Highest 6 hpi
  - PO activity- Highest 12 hpi
  - NO2- concentration- Highest 12 hpi
  - THC- Lowest 36 hpi

(Tracey et al., 1995; Huynh et al., 2011; Al-Amin et al., 2015; Al-Amin et al., 2016; Perera et al., 2017; Huynh et al., 2018; Hong et al., 2019; Park et al., 2019)

- There is an increment of *Vibrio* spp. and *Bradyrhizobium* spp. relative abundance in *Vp*AHPND-challenged *P. monodon* compared to healthy control *P. monodon* (Figure 5).
- Interestingly, the highly abundant *Maritimibacter* spp. in control sample is only detected in very less abundance for *Vp*AHPND-infected *P. monodon* (Figure 5).
- *Vp*AHPND infection not only affects the *P. monodon* gut microbiome, but also causes biochemical activity changes.

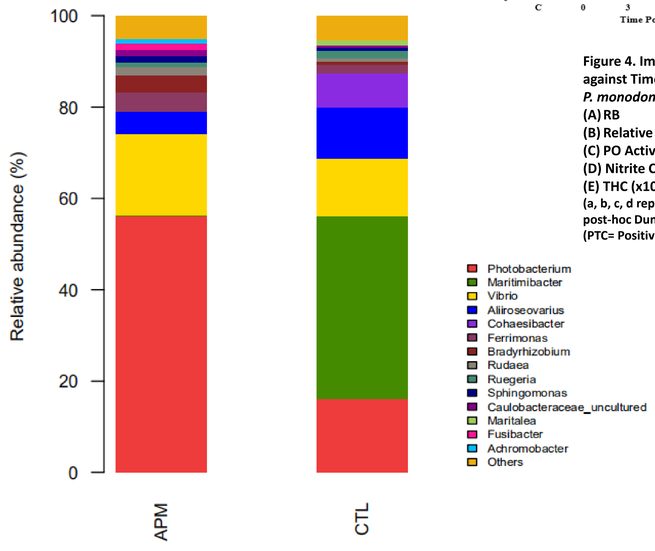


Figure 5. Relative abundance of gut microbiota (genus level) identified in 16S metagenomic analysis (CTL: Healthy control *P. monodon* gut sample, APM: *Vp*AHPND-infected *P. monodon* gut sample).

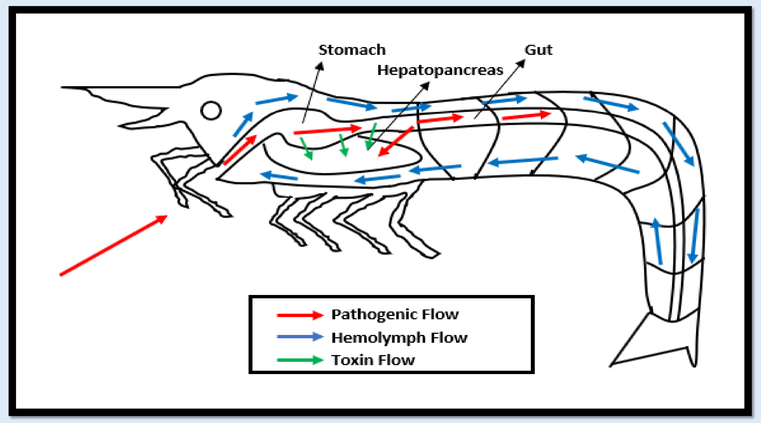


Figure 1. General outline of *Vp*AHPND infection in *P. monodon*.

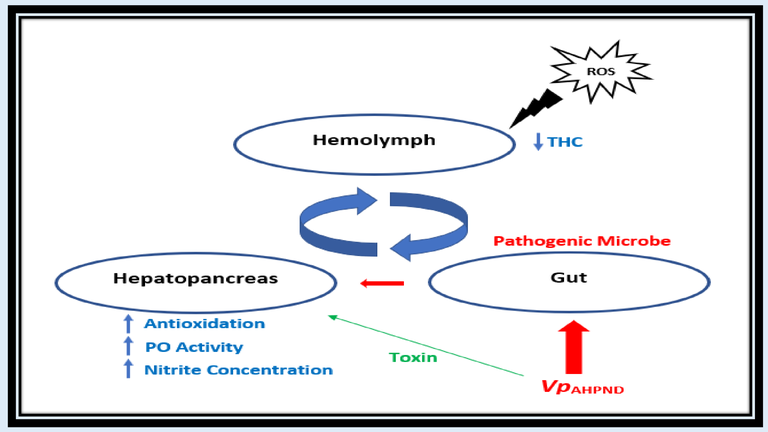
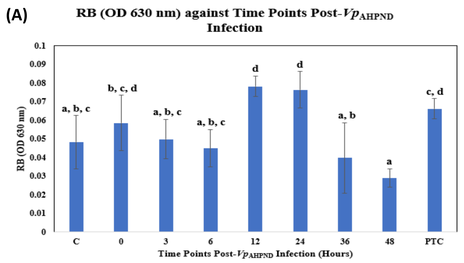
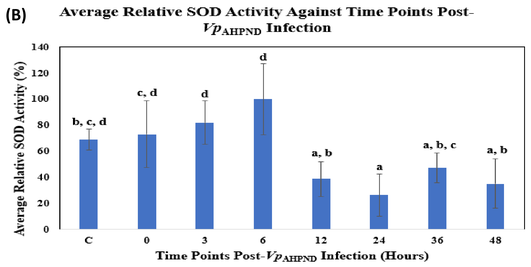


Figure 2. Postulated interactive host response of *P. monodon* towards *Vp*AHPND infection.





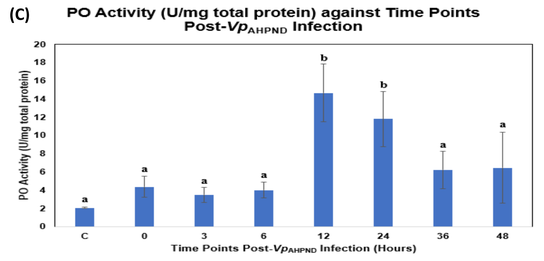
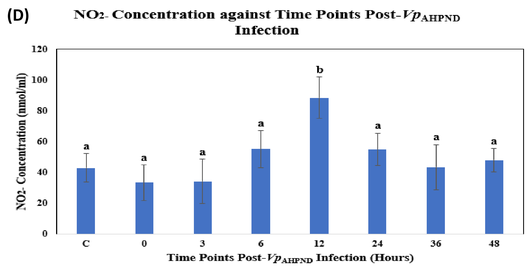
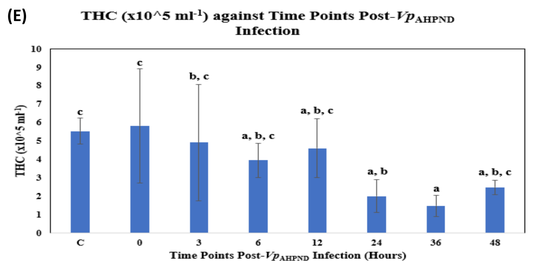


Figure 4. Immune-related biochemical changes against Time Post-*Vp*AHPND Infection (Hours) in *P. monodon* determined through:
(A) RB
(B) Relative SOD Activity
(C) PO Activity
(D) Nitrite Concentration
(E) THC (x10^5 ml^{-1})
(a, b, c, d represent different subsets of statistical post-hoc Duncan test respectively.) (C= Control) (PTC= Positive Control)





Conclusions

- The 16S metagenomic analysis between *Vp*AHPND-infected *P. monodon* and healthy control *P. monodon* samples provided insight into the host-pathogen interaction in the *P. monodon* gut during *Vp*AHPND infection.
- The immune-related biochemical changes across different post-infection time points of *Vp*AHPND-infected *P. monodon* tissue samples were identified and statistically validated.
- The identified *Vp*AHPND-influenced gut microbes or host biochemical properties could potentially be developed as biomarkers for early detection of *Vp*AHPND infection in the future with further research.

References:

1. Al-Amin, M. M., Rahman, M. M., Khan, F. R., Zaman, F., & Reza, H. M. (2015). Astaxanthin improves behavioral disorder and oxidative stress in prenatal valproic acid-induced mice model of autism. *Behavioural Brain Research*, *286*, 112-121.
2. Al-Amin, M. M., Reza, H. M., Saadi, H. M., Mahmud, W., Ibrahim, A. A., Alam, M. M., ... & Quddus, A. R. (2016). Astaxanthin ameliorates aluminum chloride-induced spatial memory impairment and neuronal oxidative stress in mice. *European Journal of Pharmacology*, *777*, 60-69.
3. Devadas, S., Bhassu, S., Soo, T. C. C., Iqbal, S. N. M., Yusoff, F. M., & Shariff, M. (2018). Draft Genome Sequence of a *Vibrio parahaemolyticus* Strain, KS17. S5-1, with Multiple Antibiotic Resistance Genes, Which Causes Acute Hepatopancreatic Necrosis Disease in *Penaeus monodon* in the West Coast of Peninsular Malaysia. *Microbiology Resource Announcements*, *7*(2), e00829-18.
4. Hong, Y., Huang, Y., Yan, G., Pan, C., & Zhang, J. (2019). Antioxidative status, immunological responses, and heat shock protein expression in hepatopancreas of Chinese mitten crab, *Eriocheir sinensis* under the exposure of glyphosate. *Fish & Shellfish Immunology*, *86*, 840-845.
5. Huynh, T. G., Cheng, A. C., Chi, C. C., Chiu, K. H., & Liu, C. H. (2018). A synbiotic improves the immunity of white shrimp, *Litopenaeus vannamei*: Metabolomic analysis reveal compelling evidence. *Fish & Shellfish Immunology*, *79*, 284-293.
6. Huynh, T. G., Yeh, S. T., Lin, Y. C., Shyu, J. F., Chen, L. L., & Chen, J. C. (2011). White shrimp *Litopenaeus vannamei* immersed in seawater containing *Sargassum hemiphyllum* var. chinense powder and its extract showed increased immunity and resistance against *Vibrio alginolyticus* and white spot syndrome virus. *Fish & Shellfish Immunology*, *31*(2), 286-293.
7. Park, K., Kim, W. S., & Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, *Macrophthalmus japonicus*. *Fish & Shellfish Immunology*, *87*, 322-332.
8. Perera, N. C. N., Godahewa, G. I., Lee, S., Kim, M. J., Hwang, J. Y., Kwon, M. G., ... & Lee, J. (2017). Manganese-superoxide dismutase (MnSOD), a role player in seahorse (*Hippocampus abdominalis*) antioxidant defense system and adaptive immune system. *Fish & Shellfish Immunology*, *68*, 435-442.
9. Tracey, W. R., Tse, J., & Carter, G. (1995). Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. *Journal of Pharmacology and Experimental Therapeutics*, *272*(3), 1011-1015.

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