Effects of *Bacillus amyloliquefaciens* and *Exiguobacterium aestuarii* on the Physiological and Toxicological Responses of the Pacific White Shrimp (*Litopenaeus vannamei*) Under Acute Diazinon Exposure

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Abstract

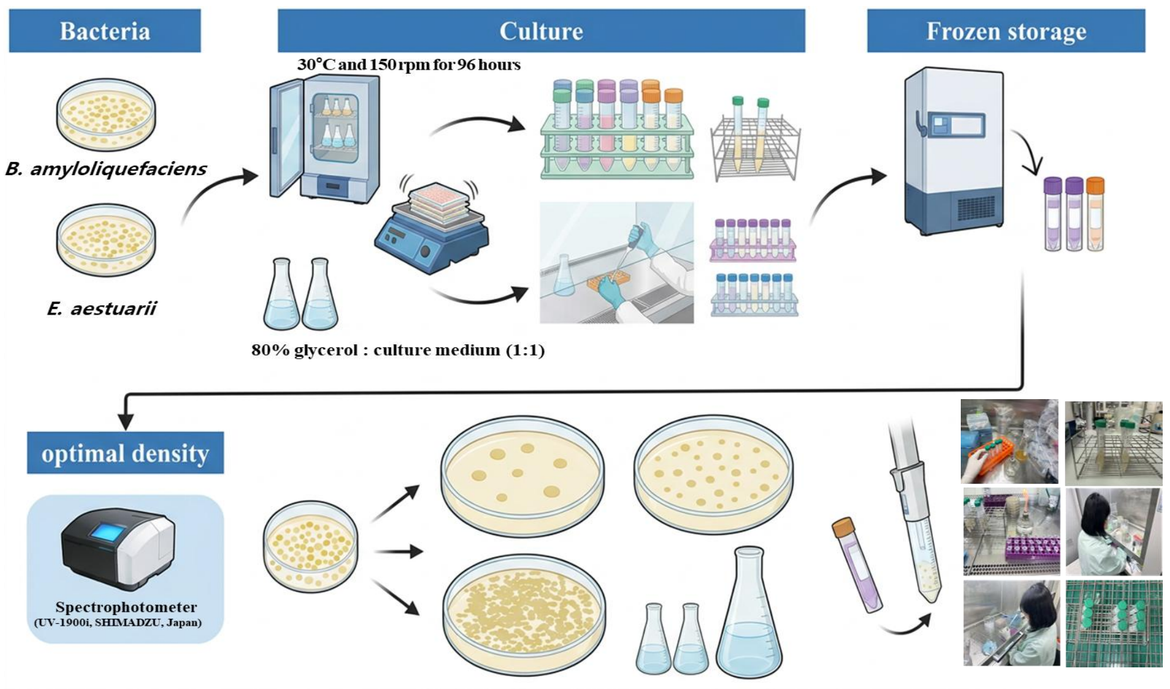
- This study aims to mitigate the aquatic toxicity of diazinon, a persistent organophosphate insecticide, using degrading microorganisms. *Bacillus amyloliquefaciens* and *Exiguobacterium aestuarii* were identified as highly efficient strains, and their mixed culture rapidly degraded approximately 72% of diazinon. While diazinon exposure induced oxidative stress and neurotoxicity (inhibiting AChE activity) in whiteleg shrimp, the microbial treatment significantly reduced oxidative stress and stabilized AChE activity. Overall, the mixed culture of *B. amyloliquefaciens* and *E. aestuarii* effectively enhances diazinon degradation and mitigates its toxic effects, demonstrating strong potential for aquatic bioremediation.

Introduction

- Excessive use of organophosphate insecticides causes aquatic contamination and toxic effects on non-target organisms. Specifically, persistent diazinon significantly impairs the neurological and physiological functions of the Pacific white shrimp (*L. vannamei*). This study investigates the impact of acute diazinon exposure on the survival and toxicological responses of this major aquaculture species. The findings provide a critical basis for assessing and managing the environmental risks posed by insecticide contamination.

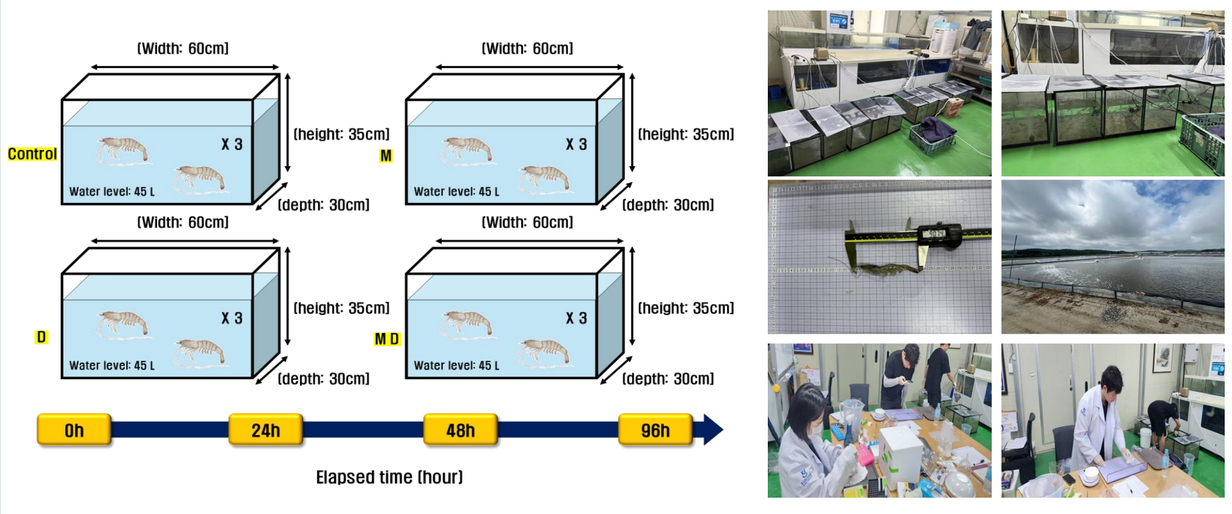
Material and Methods

● Microbial Growth and Cultivation



- Cultured in MSM medium with diazinon (30 mg/L) at 30°C and 180 rpm for 96 hours.
- Optical density at 600 nm was measured at 0, 24, 48, 72, and 96 hours.

● Acute Toxicity Test



- Rectangular tanks : glass tank, 35 × 60 × 35 cm, 74 L).
- Shrimp (TL 8.4±0.8 cm, BW 4.2±0.1 g).
- Density : 50 shrimp /tank.

Results

● Acute Toxicity and LC_{50} Determination

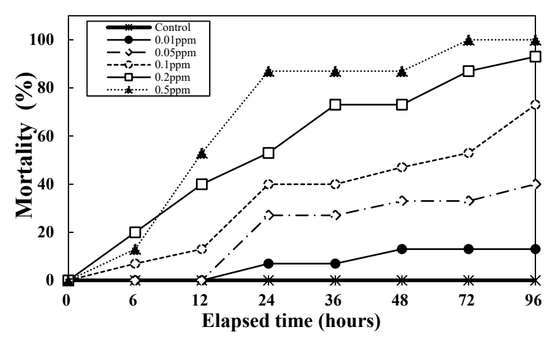


Fig. 1. Mortality rate of *L vannamei* individuals after 96 hours of exposure to five concentrations (0.01, 0.05, 0.1, 0.2, 0.5 mg/L) of diazinon .

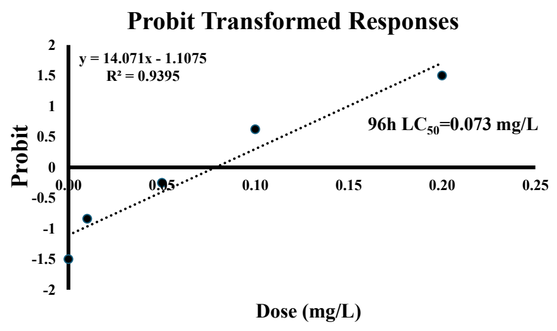


Fig. 2. Probit-transformed dose-response curve for the 96-hour acute toxicity of *L. vannamei* exposed to diazinon concentrations.

● Enzyme Activities / AChE Activitiy

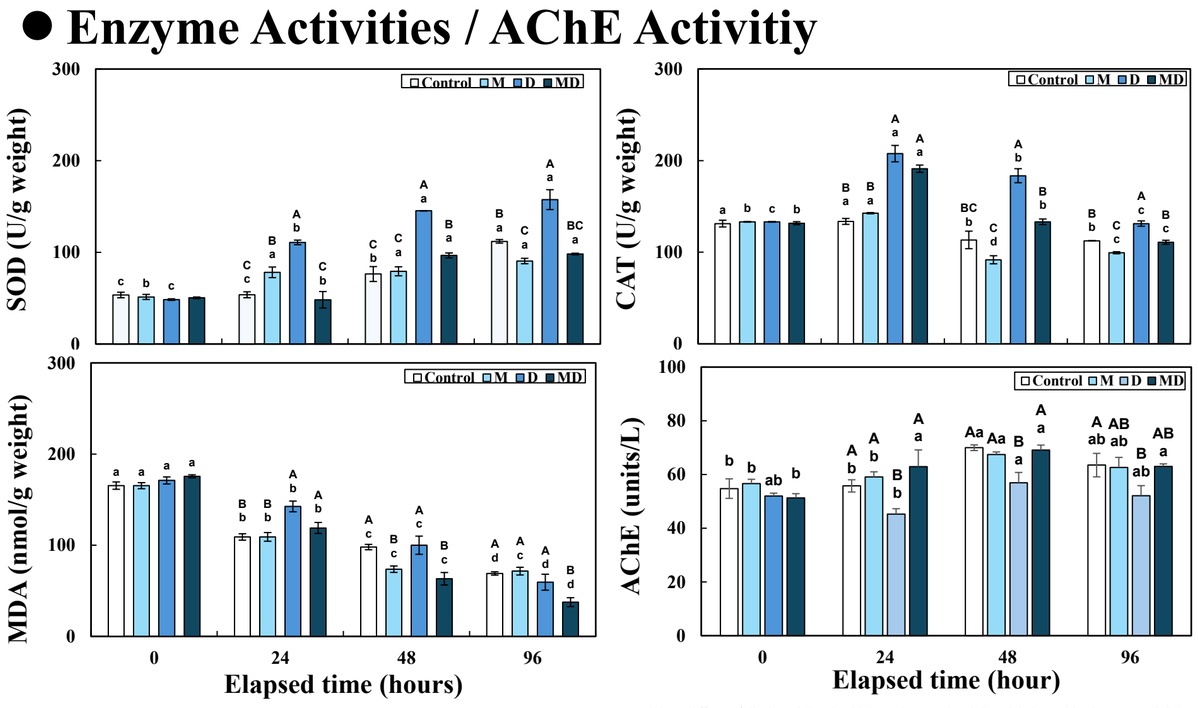


Fig. 3. Effects of diazinon (D), microbial supplementation (M) and their combined treatment (MD) on antioxidant enzyme activities (SOD, CAT and MDA) in the hepatopancreas of *L. vannamei* over 96 hours (0, 24, 48, and 96 h). Values are presented as mean±standard error (mean±SE). Capital letters indicate significant differences among treatments at the same time point, while lowercase letters indicate significant differences over time within the same treatment ($p<0.05$).

Fig. 4. Effects of diazinon (D), microbial supplementation (M) and their combined treatment (MD) on AChE activity in the brain of *L. vannamei* over 96 hours (0, 24, 48 and 96 h). Values are presented as mean±standard error (mean±SE). Capital letters indicate significant differences among treatments at the same time point, while lowercase letters indicate significant differences over time within the same treatment ($p<0.05$)

● Gene Expression

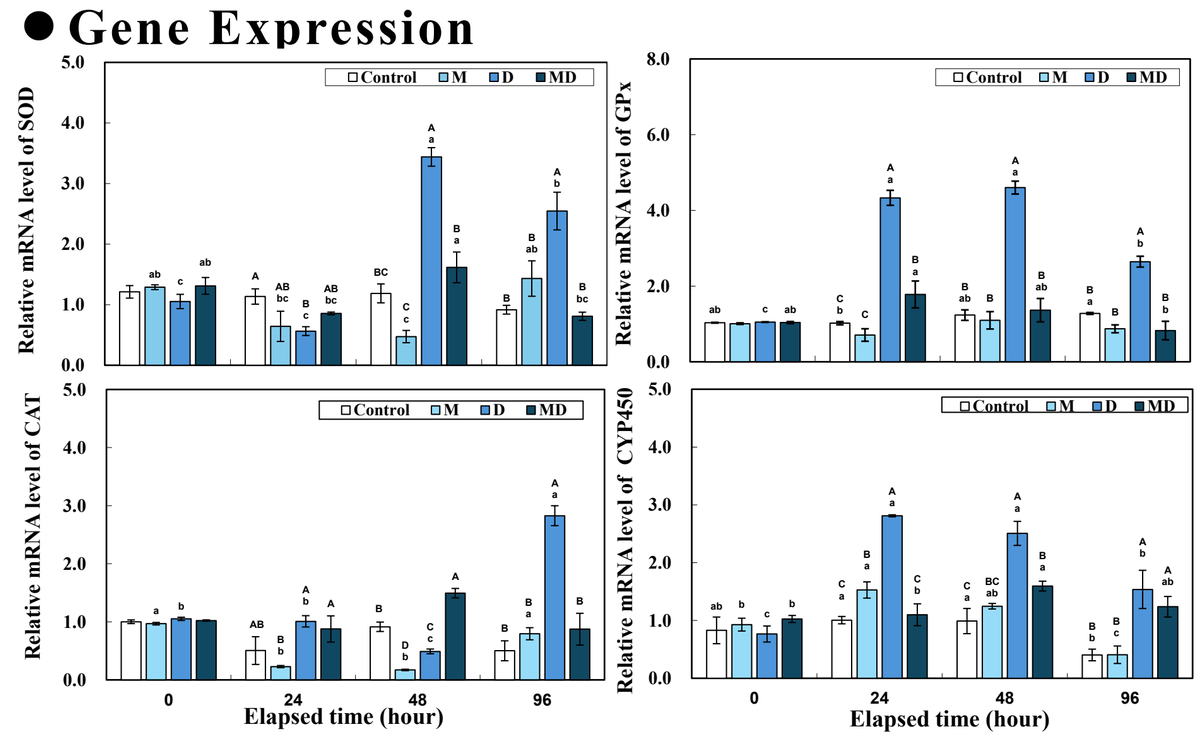


Fig. 5. Effects of diazinon (D), microbial supplementation (M), and their combined treatment (MD) on the gene expression of SOD, CAT, GPx and CYP450 in the hepatopancreas of *L. vannamei* over 96 hours (0, 24, 48 and 96 h). Values are presented as mean±standard error (mean±SE). Capital letters indicate significant differences among treatments at the same time point, while lowercase letters indicate significant differences over time within the same treatment ($p<0.05$)

Summary and conclusion

1) These results demonstrate that the co-culture of *B. amyloliquefaciens* and *E. aestuarii* effectively alleviates diazinon-induced oxidative stress and neurotoxicity while supporting biodegradation.
2) Future studies should establish precise bioremediation strategies by analyzing specific microbial degradation pathways and molecular responses of antioxidant genes.