

Investigating the Impact of Quercetin on the Antimicrobial Resistance Traits of Shrimp against *Vibrio parahaemolyticus* by Transcriptomic and Metabolomic Analyses

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Abstract: Shrimp is an important aquaculture species worldwide. *Vibrio parahaemolyticus* (VP) is an opportunistic pathogen of *Litopenaeus vannamei* that can cause diseases such as acute hepatopancreatic necrotic disease (AHPND), resulting in significant losses to the shrimp farming industry. This study aimed to explore the factors contributing to variation in the antimicrobial resistance traits of diverse populations of *Litopenaeus vannamei* in response to VP. Comprehensive analyses of transcriptome and metabolome data were performed. The findings demonstrated that flavonoid compounds, especially quercetin, play a pivotal role in the variation in the antimicrobial resistance traits of Mx and TH04 populations. Supplementing feed with an appropriate quantity of quercetin has the potential to increase the expression of crucial genes in the NF- κ B pathway, including TLR and AP1, along with the expression of the antibacterial peptide crustin, resulting in a decreased mortality rate. Together, these results indicate that an appropriate amount of quercetin can strengthen the immune response of shrimp to VP, thereby reducing the incidence of AHPND.

Method: Six different shrimp populations were selected, including TH04, Mx, TL, MN, CT, and MO, for VP infection experiments. The final injection density of VP was adjusted to yield approximately 2.5×10^7 CFU/50 μ L. On the 5th day (120 h) after infection, all shrimp were collected and marked to estimate cumulative mortality. Based on the survival rate curves, the Mx population with the highest survival rate and the TH04 population with the lowest survival rate were chosen for transcriptional and metabolome sample collection and sequencing.

Feeding trial

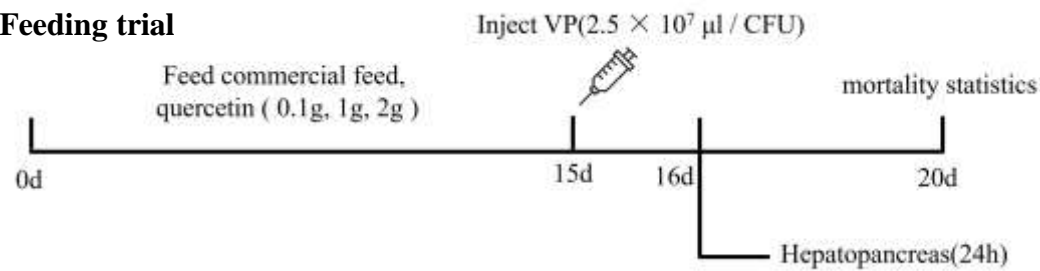


Fig. 1 Course of the quercetin feeding experimental program.

Results

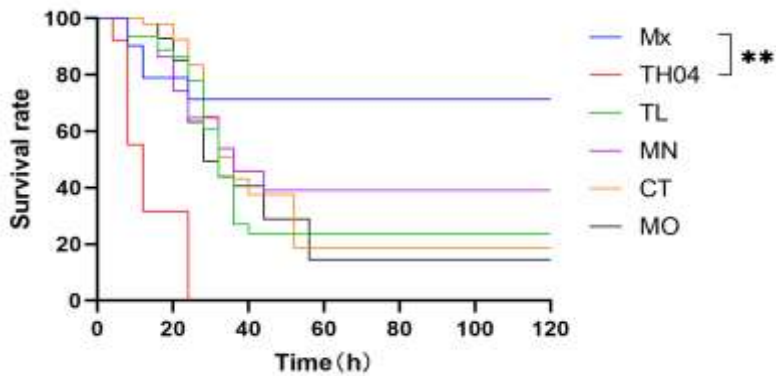


Fig. 2 Survival rates of six *L. vannamei* populations infected with VP.

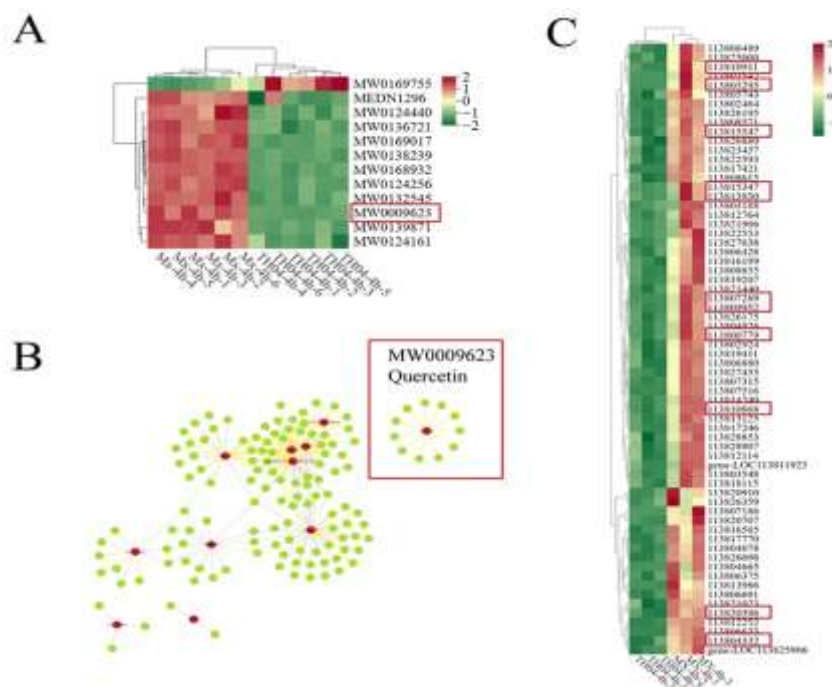


Fig. 3 Correlation analysis of metabolomic and transcriptomic data. (A) Metabolomic analysis of heat maps of DMs associated with flavonoids in the TH04 and Mx populations (quercetin is in the red box). (B) Network diagram of genes associated with flavonoids (genes associated with quercetin are in red boxes). Solid yellow lines indicate positive correlations and dashed grey lines indicate negative correlations; red circles are metabolites and green circles are genes. (C) Transcriptomics analysis of heat maps of DEGs associated with flavonoids in the TH04 and Mx populations.

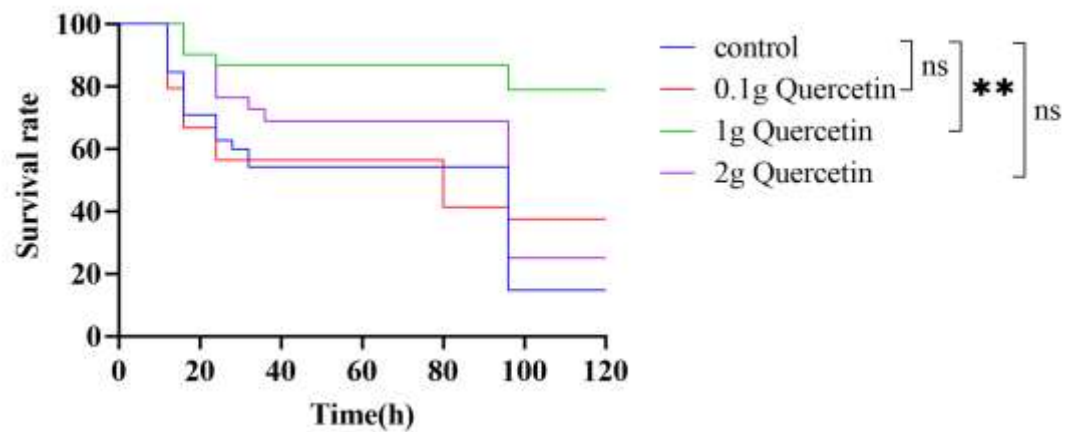


Fig. 4 Survival of the different experimental groups quercetin on *L. vannamei* infected with VP.

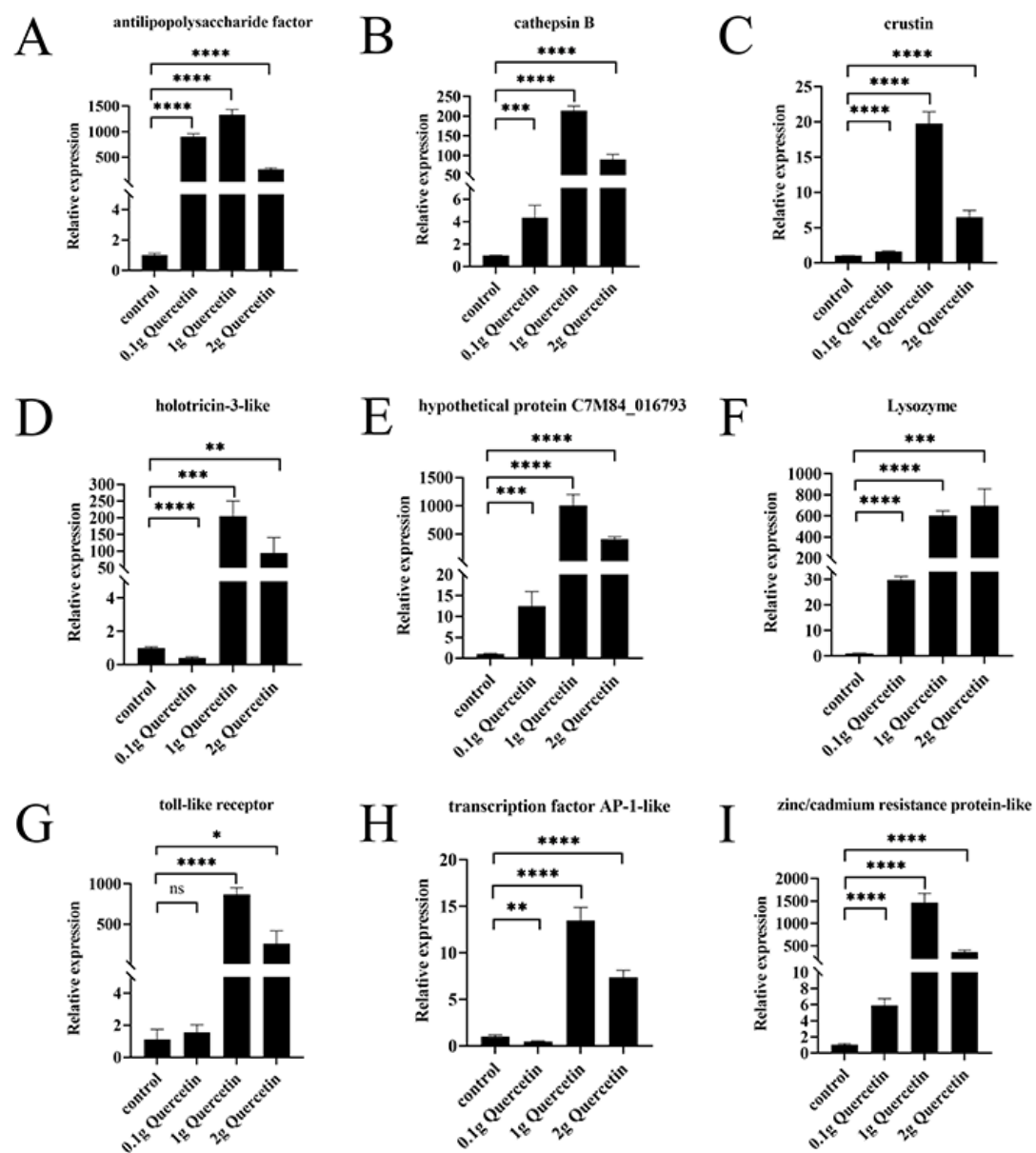


Fig. 5 Gene expression of immune-related factors in *L. vannamei* after feeding with different levels of quercetin and challenge with *Vibrio*. (A) Anti-lipopolysaccharide factor gene, (B) cathepsin B, (C) crustin, (D) holotricin-3-like, (E) hypothetical protein C7M84_016793, (F) lysozyme, (G) Toll-like receptor, (H) transcription factor AP-1-like, and (I) zinc/cadmium resistance protein-like in the hepatopancreas of *L. vannamei* infected with VP for 24 h, under the action of different concentrations of quercetin.

Conclusions: 1. In this study, a joint analysis of the transcriptome and metabolome data were used to investigate the reasons for phenotypic differences in resistance to *Vibrio parahaemolyticus* (VP) in *Litopenaeus vannamei*. The identification of flavonoid compounds, especially quercetin, play a critical role in influencing the *anti-Vibrio parahaemolyticus* characteristics of shrimp. 2. Feeding shrimps with quercetin can enhance their antibacterial immune ability. Adding 1 g of quercetin per 100 g of feed can significantly increase the activity of the NF- κ B pathway in shrimps, strengthen their resistance against *Vibrio parahaemolyticus*, and reduce mortality.

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