Study of JNK Pathway Transcription Factor *TmKayak* Gene in *Tenebrio molitor*

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Abstract

Insect innate immune responses are activated by several signaling pathways such as Toll, immune deficiency (IMD), Jun N-terminal kinase (JNK), and Janus kinase/signal transducers (JAK/STAT). Jun N-terminal kinase (JNK) is one of the mitogen-activated protein kinases and regulates a number of cellular processes. Kayak is one of the JNK-activated transcription factors. JNK pathway studies in *Tenebrio molitor* model are largely unknown. In this study, using RNAi experiment, we demonstrated that *TmKayak* regulated antimicrobial peptide expression. First, we identified *Kayak* gene by analysis of sequence information generated from EST and RNA sequencing data in *Tenebrio molitor*. Temporal and spatial expression patterns of *TmKayak* mRNA were analyzed with late instar larvae and 5-day-old adults of *T. molitor*. *TmKayak* expression levels were examined at 3, 6, 9, 12, and 24 h in different tissues and the whole body after infection with *E. coli*, *S. aureus* and *C. albicans*. To know the immunological role of *TmKayak*, RNAi technique was used to silence *TmKayak* gene. Functional loss of *TmKayak* has distinct effect on the survival of *T. molitors* infected *E. coli*, *S. aureus*, and *C. albicans*. Also, this functional loss reduced mRNA expression of antimicrobial peptide in all organ which we observed except hemocytes. Conclusionally, *TmKayak* is required for *T. molitor* defense against Gram negative, positive bacteria and fungus.

Keywords: JNK pathway, *Tm*Kayak, *Tenebrio molitor*, antimicrobial peptides, microbial challenge

Results

1. Effect of *TmKayak* Knockdown on the survivability of *Tenebrio molitor*

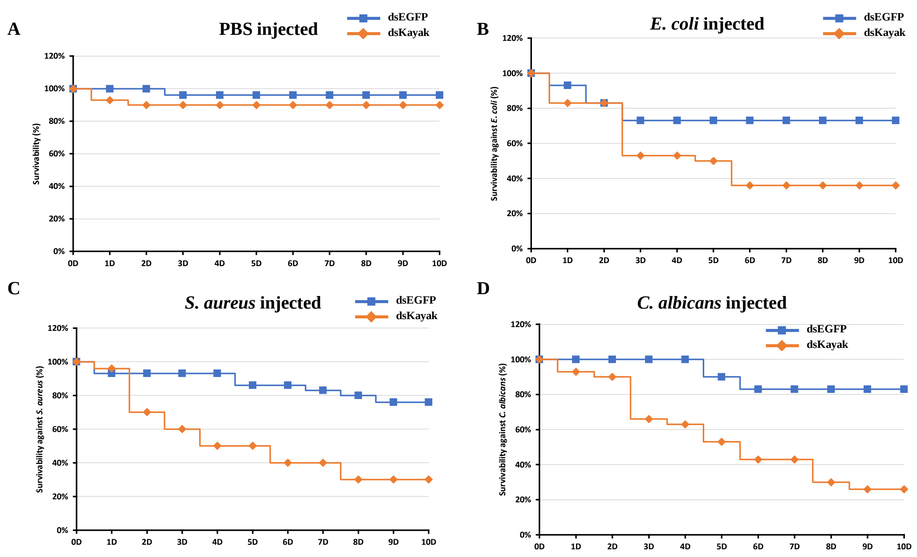

Figure 1. Effect of *TmKayak* Knockdown on the survivability of *Tenebrio molitor*
(A)*TmKayak* knockdown efficiency measured using quantitative real-time polymerase chain reaction at day 5 post injection. Viability of *TmKayak* knockdown larvae after challenge with *Escherichia coli* (B), *Staphylococcus aureus* (C), or *Candida albicans* (D) (n = 30).

2. AMP encoding mRNA expression patterns in Fat body

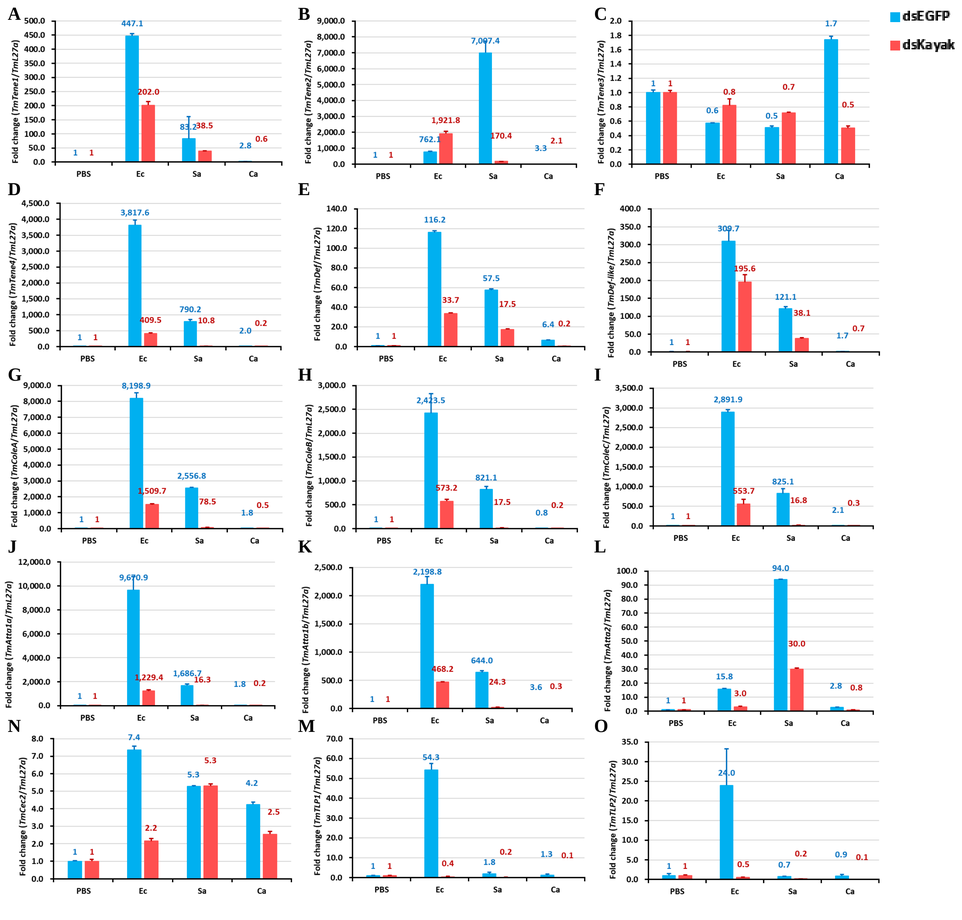

Figure 2. Antimicrobial peptide (AMP)-encoding mRNA expression patterns in fatbody of *TmKayak* knockdown larvae in response to *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* infections.
At 12 h post-microbial challenge, the expression levels of AMP-encoding genes, including TmTene1 (A), TmTene2 (B), TmTene3 (C), TmTene4 (D), TmDef (E), TmDef-like (F), TmCec2 (G), TmColeA (H), TmColeB (I), TmColeC (J), TmAtt1a (K), TmAtt1b (L), TmAtt2 (M), TmTLP1 (N), and TmTLP2 (O), were measured using quantitative real-time polymerase chain reaction. dsEGFP was injected as a negative control.

Conclusion

The expression of the *TmKayak* transcript in the ds*TmKayak*-injected larvae decreased by approximately 80% compared with the control group (ds*EGFP*) at day 5 post-injection. The survivability of *TmKayak*-silenced larvae exposed to *E. coli* showed less than 40% viability. The viability rates significantly decreased at day 3 post-*E. coli* infection. The viability rates of *S. aureus* and *C. albi cans*-infected *TmKayak*-silenced were less than 30% viability. The viability rates significantly decreased at day 2 post-*S. aureus* and day 3 post-*C. albicans* infection. These results suggested that knockdown of *TmKayak* increase lethality of Gram-negative, Gram-positive and fungal infected *T. molitor* larvae. Among 15 AMP genes examined, the 12 genes were significantly downregulated in the *E. coli*, *S. aureus*, and *C. albicans* infected *Tmkayak* knockdown larvea fatbody. Among 15 AMP genes examined, the 13 genes were significantly downregulated in the *E. coli*, *S. aureus*, and *C. albicans* infected *Tmkayak* knockdown larvea Malpighian tubules. Among 15 AMP genes examined, the 7 genes were significantly downregulated in the *E. coli*, *S. aureus*, and *C. albicans* infected *Tmkayak* knockdown larvea whole body. These result supported viability of *TmKayak* knockdown individuals.

3. AMP encoding mRNA expression patterns in Malpighian tubules

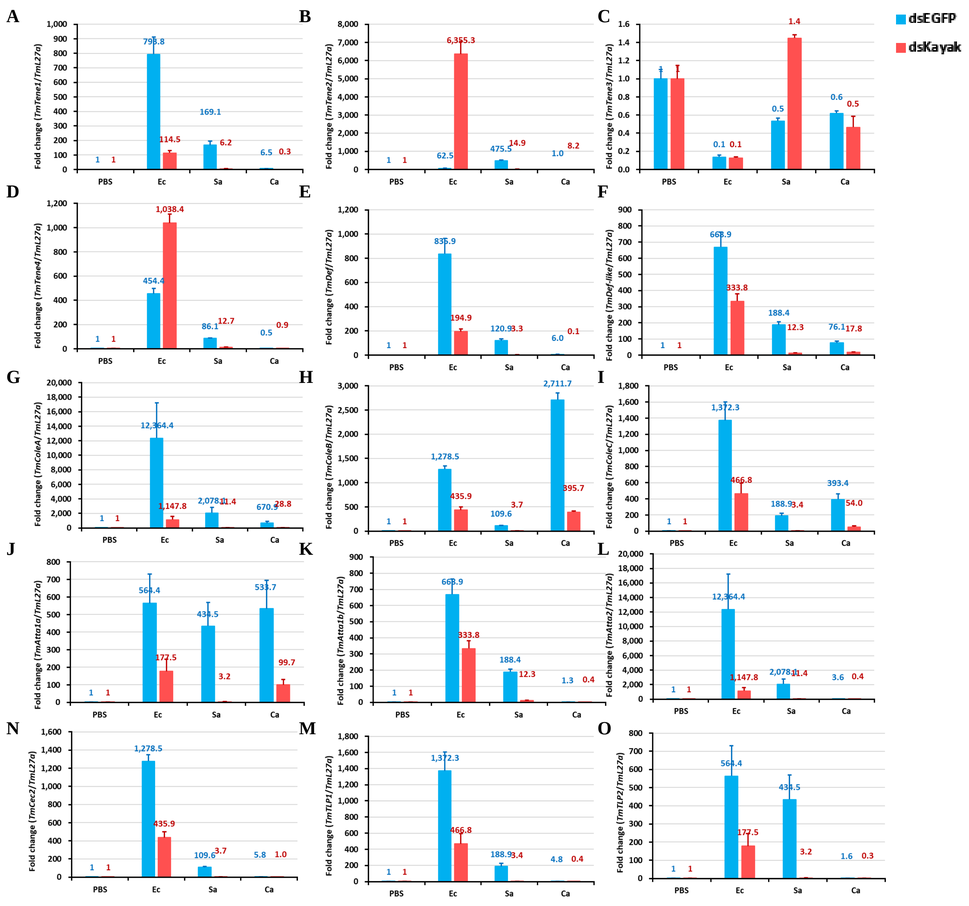

Figure 3. Antimicrobial peptide (AMP)-encoding mRNA expression patterns in Malpighian tubules of *TmKayak* knockdown larvae in response to *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* infections.
At 12 h post-microbial challenge, the expression levels of AMP-encoding genes, including TmTene1 (A), TmTene2 (B), TmTene3 (C), TmTene4 (D), TmDef (E), TmDef-like (F), TmCec2 (G), TmColeA (H), TmColeB (I), TmColeC (J), TmAtt1a (K), TmAtt1b (L), TmAtt2 (M), TmTLP1 (N), and TmTLP2 (O), were measured using quantitative real-time polymerase chain reaction. dsEGFP was injected as a negative control.

4. AMP encoding mRNA expression patterns in Whole body

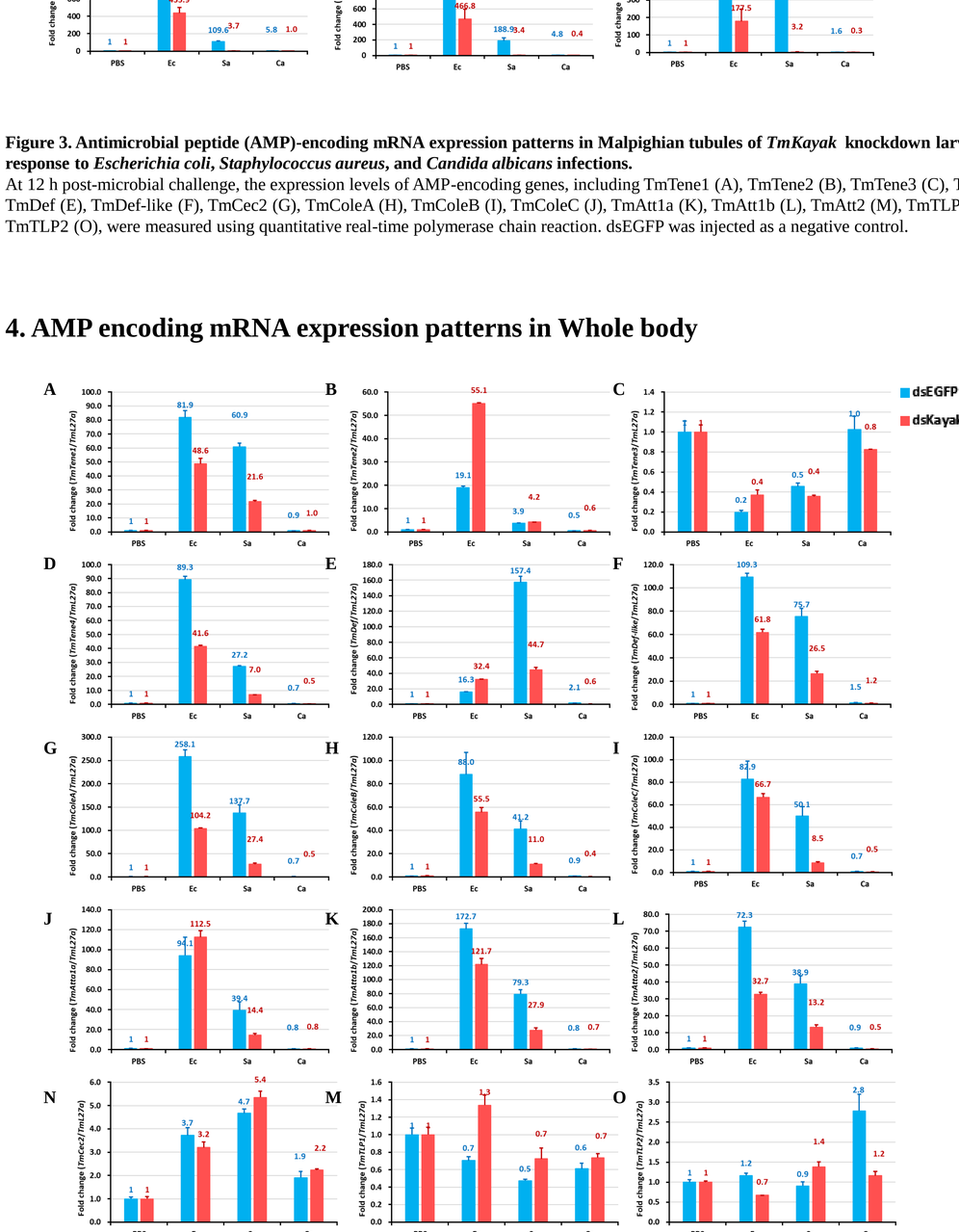

Figure 4. Antimicrobial peptide (AMP)-encoding mRNA expression patterns in Whole body of *TmKayak* knockdown larvae in response to *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* infections.
At 12 h post-microbial challenge, the expression levels of AMP-encoding genes, including TmTene1 (A), TmTene2 (B), TmTene3 (C), TmTene4 (D), TmDef (E), TmDef-like (F), TmCec2 (G), TmColeA (H), TmColeB (I), TmColeC (J), TmAtt1a (K), TmAtt1b (L), TmAtt2 (M), TmTLP1 (N), and TmTLP2 (O), were measured using quantitative real-time polymerase chain reaction. dsEGFP was injected as a negative control.