Effects of *TmTak1* gene silencing on expression patterns of anti-microbial peptides (AMPs) in *Tenebrio molitor*

Su Hyeon Hwang [p1], Keunho Yun [1], Maryam Ali Mohammadie Kojour [1]
Ho Am Jang[2], Youg Seok Lee [2], Yong Hun Jo [c1], and Yeon Soo Han [c1]

[1]Department of Applied Biology, Institute of Environmentally-Friendly Agriculture (IEFA), College of Agriculture and Life Sciences, Chonnam National University, Gwangju, 61186, Republic of Korea
[2]Department of Biology, College of Natural Sciences, Soonchunhyang University, Asan City 31538, Republic of Korea

Abstract

Previous reports showed that insects have two major immune signaling cascade s (Toll and IMD) for producing antimicrobial peptides(AMP) in responses to various pathogens in many insect models. The IMD pathway is initiated by peptidoglycan recognition proteins (PGRPs) that recognize PGN of Gram-negative bacteria, and finally produces AMPs. It has been known that Tak1 plays an important role in phosphorylating the IKK complex i n the IMD pathway during the host-pathogen interactions in insects. However, no functional characterization of *TmTak1* in *Te nebrio molitor* has so far been reported.
Analysis of temporal expression patterns showed that the highest expression lev el of *TmTak1* mRNA was detected at young larvae (YL), but the lowest express ion levels were observed at both larval-pupal transition stage (PP) and pupal-adult transition stage (P7) just before emergence. Spatial expression analysis indicate d that *TmTak1* mRNA is highly expressed in the larval gut, but mainly expresse d in the fat body in *T. molitor*. Interestingly, induction pattern analysis revealed that the highest expression level (20 times at 6 h post infection) was detected in Malpighian tubules when *T. molitor* larvae were immune challenged with a human pathogen, *Candida albicans*. Most interestingly, analy sis of AMPs expression patterns in whole body after *TmTak1* gene silencing sho wed that most of the AMP mRNAs including TmTenecin-1, -2, -4, TmDefencin, TmDefencin-like, TmColeoptericin-A, -B, -C, TmAttacin-1a, -1b, 2, and TmThaumatin-like 1 were down-regulated except TmTenecin-3, TmCecropin-2 and TmTLP2.
Taken together, it suggests that TmTak1 may play a critical role in innate immune responses in *T. molitor*.

Keywords: IMD pathway, Tak1, *Tenebrio molitor*, RNAi antimicrobial peptides

Conclusion

For *TmTak1*, the highest expression levels were seen in the Malpighian tubules at 6h after fungal infection. Among 15 AMP genes examined, the 12 genes were significantly downregulated in the *E. coli*, *S. aureus*, and *C. albicans* infected *TmTak1* knockdown larvea whole body. These result will support viability of *TmKayak* knockdown individuals.

Results

1. *TmTak1* mRNA expression level after microbial challenge

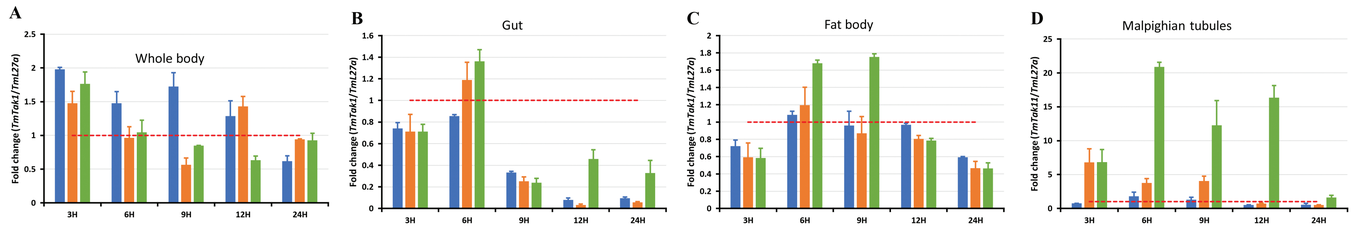

Figure 1. *TmTak1* mRNA expression profiles after microbial challenge.
Expression of *TmTak1* mRNA in the whole body (A), gut (B), fat body (C), Malpighian tubules (D) of larvae infected with *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.

2. *TmTak1* AMP expression

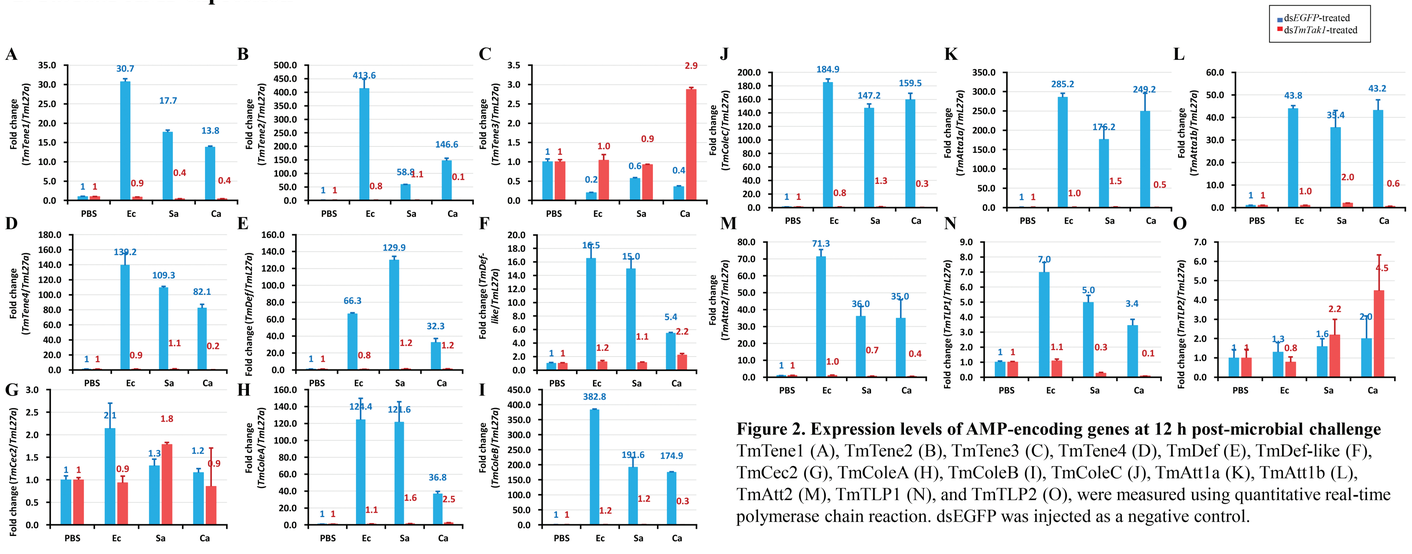

Figure 2. Expression levels of AMP-encoding genes at 12 h post-microbial challenge
TmTene1 (A), TmTene2 (B), TmTene3 (C), TmTene4 (D), TmDef (E), TmDef-like (F), TmCec2 (G), TmColeA (H), TmColeB (I), TmColeC (J), TmAtt1a (K), TmAtt1b (L), TmAtt2 (M), TmTLP1 (N), and TmTLP2 (O), were measured using quantitative real-time polymerase chain reaction. dsEGFP was injected as a negative control.