TmFADD Plays Important Role in Response to Gram-negative Bacteria by Regulating Antimicrobial Peptides (AMPs) as an Adaptor Protein of Imd Pathway in *Tenebrio molitor*

Keunho Yun [p1], Ho Am Jang [2], Su Hyeon Hwang [1], MaryamAli Mohammadie Kojour [1], Yong Seok Lee [2], Yong Hun Jo [c2] 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, Korea

Abstract

Fas-associated death domain protein (FADD) was first identified for its critical function in apoptosis in mammalians. Furthermore, several studies in arthropods have revealed that FADD, forming a complex with IMD and DREADD, plays a role at the beginning of the immune deficiency (IMD) pathway. However, the sequence and its role of FADD in *Tenebrio molitor*, the mealworm beetle, have not been discovered yet. Herein, we discovered and cloned the designated FADD sequence of *Tenebrio molitor*. To further elucidate the specific role of TmFADD in *Tenebrio mollitor* upon microbial infection, we designed RNA interference technology and tested microbial challenge upon *Escherichia coli(E. coli), Staphylococcus aureus(S. aureus)*, and *Candida albicans(C, albicans)* infections. Designed *TmFADD*-targeting dsRNA downregulated *TmFADD* mRNA level by 30% 6 days after injection. For the microbial challenge test, we also infected *E. coli, S. aureus*, or *C. albicans* to each group of *Tenebrio molitor* larvae whose *TmFADD* expression was reduced. Among the infected microbes, we found that *E.coli* injected group showed a significantly decreased survival rate of less than 30%. To further understand the unknown mechanism of the selective vulnerability against gram-negative bacteria, we analyzed the molecular markers of the IMD pathway, one of the representative antibacterial immune systems in *Tenebrio molitor*. In the *E.coli* infected group, the expression of antimicrobial peptide (AMP) and NF-kB transcription factor genes were increased even though *TmFADD* was downregulated. These results suggest that *TmFADD* may play another role against gram-negative bacteria independently of the IMD pathway, unlike in other arthropods.

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

Conclusion

The expression of the *TmFADD* transcript in the ds*TmFADD*-injected larvae decrea sed by approximately 70% compared with the control group (ds*EGFP*) at day 6 pos t-injection. The survivability of *TmFADD*-silenced larvae exposed to *E. coli* showed less than 60% viability. The viability rates significantly decreased at day 2 post-*E. c oli* infection. These results suggested that knockdown of *TmFADD* increase lethality of Gram-negative infected *T. molitor* larvae. Among 15 AMP genes examined, the 13 genes were significantly upregulated in the *E. coli*, *S. aureus*, and *C. albicans* infected *TmFADD* knockdown larvea whole body. These result did not support viability of *TmFADD* knockdown individuals.

Results

1. Mortality of *TmFADD* knocked-down *T. molitor* after microbial challenge.

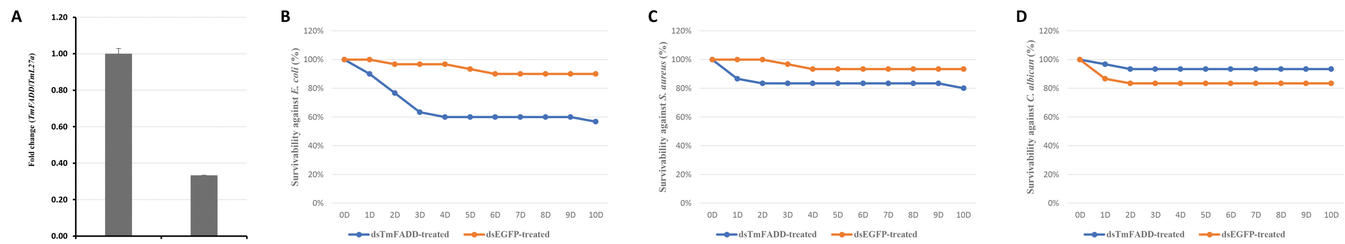

Figure 1. Effects of *TmFADD* RNAi on *T. molitor* larval mortality against microbial challenges.
TmFADD RNAi efficiency in *T. molitor* larvae (A) and mortality of *T. molitor* larvae against *Escherichia coli* (B), *Staphylococcus aureus* (C), and *Candida albicans* (D). 30 larvae were examined in each group. ds*EGFP*-treated group was also injected with same microbes for negative control.

2. mRNA expression patterns of antimicrobial peptide (AMP) and NF-kB transcription factors.

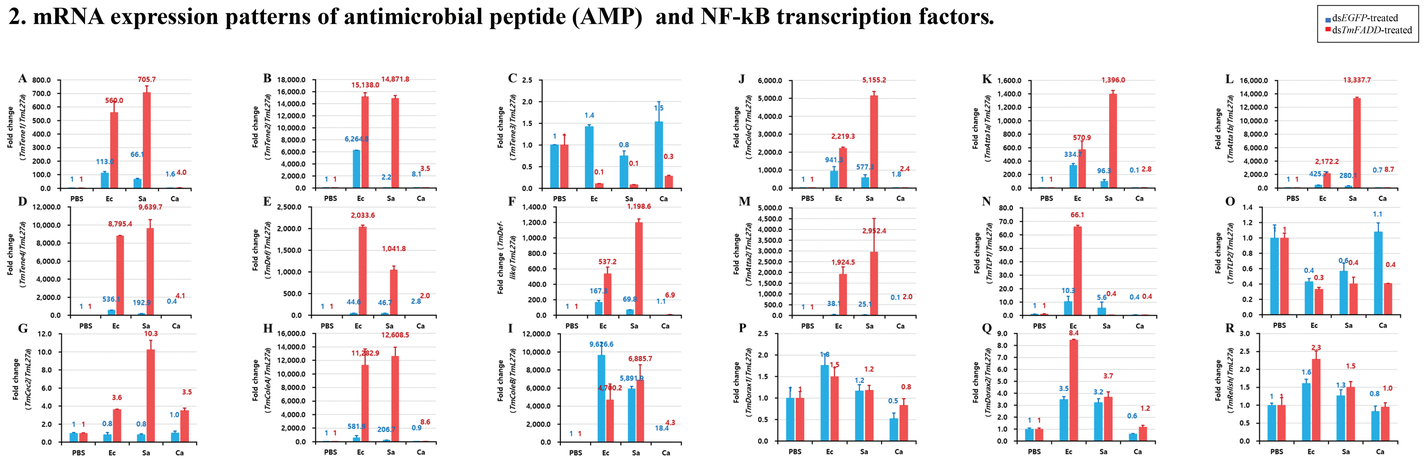

Figure 2. Antimicrobial peptide (AMP) genes mRNA expression patterns.
The whole body of *Tenebrio molitor* larvae were collected after 24 h. Phosphate-buffered saline (PBS) was used as a control. The expression level of TmTene1 (**A**), TmTene2 (**B**), TmTene3 (**C**), TmTene4 (**D**), TmDef (**E**), TmDef-like (**F**), TmCec2 (**G**), TmColeA (**H**), TmColeB (**I**), TmColeC (**J**), TmAtt1a (**K**), TmAtt1b (**L**), TmAtt2 (**M**), TmTLP1 (**N**), TmTLP2 (**O**) TmDorax1 (**P**), TmDorax2 (**Q**), and TmRelish (**R**) were quantified with quantitative real-time polymerase chain reaction (RT-qPCR). dsEGFP was used as a knock-down control and TmL27a (Tenebrio molitor ribosomal protein) was used as an internal control. The numbers and the bars indicate the expression levels of each group. The blue bars represent the dsEGFP-treated groups and the red bars represent the dsTmFADD-treated groups. 20 larvae of T. molitor were examined in each group.