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Fatty acid synthase plays a positive role in shrimp immune responses against *Vibrio parahaemolyticus* infection

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ABSTRACT

Fatty acid synthase (FAS) is an important enzyme that catalyzes the synthesis of fatty acids. In this study, the role of the FAS gene from pacific white shrimp *Litopenaeus vannamei* (LvFAS) in immune responses against *Vibrio parahaemolyticus* infection was studied. The expression of LvFAS could be up-regulated upon infection of *V. parahaemolyticus* and stimulation of lipopolysaccharide and poly (I:C). The promoter of LvFAS was predicted to harbor a NF-κB binding site and dual-luciferase reporter assays demonstrated that the NF-κB family proteins Relish, sRelish and Dorsal could activate the transcription of LvFAS. After knockdown of LvFAS expression using RNAi strategy, both the mortality of *V. parahaemolyticus* infected shrimps and the bacterial load in shrimp tissues were significantly increased. Meanwhile, the expression of many immune-responsive genes, such as antimicrobial peptides, C-type lectins (CTLs), lysozyme and hemolin, was down-regulated. These suggested that LvFAS could play a positive role in *anti-V. parahaemolyticus* responses in shrimp. To our knowledge, this is the first study that investigates the role of FAS in antibacterial immunity in animals, which may indicate the relationship between the anabolism of fatty acids and immune responses in crustaceans.

1. Introduction

White pacific shrimp, *Litopenaeus vannamei*, belonging to Penaeidae family of decapod crustaceans, is the major aquaculture shrimp species in the world, whose production accounts for more than two-third of the global shrimp aquaculture production every year [1]. The shrimp farming industry is threatened by a wide range of pathogens, in particular bacteria and viruses, which have caused serious economic losses [2]. In recent 20 years, more and more studies have been focused on the immune responses of *L. vannamei*. A series of antibacterial and antiviral mechanisms of *L. vannamei* have been systemically studied, establishing *L. vannamei* as a useful model for studying the invertebrate immune system [3–5].

Fatty acid synthase (FAS) is an enzyme playing a crucial role in lipid metabolism [6]. It functions to catalyze the *de novo* synthesis of palmitate, a fatty acid for synthesis of more complex fatty acids and plasma membrane structure, by using acetyl-CoA and malonyl-CoA as substrates and NADPH as electron donor [7,8]. The *L. vannamei* FAS gene (LvFAS, Genbank accession No. HM595630) has been identified, which encodes an protein of 2509 amino acid residues and shares similarities with insect and mammalian FAS [9]. Interestingly, the expression of LvFAS was up-regulated in white spot syndrome virus (WSSV)-infected tissues, suggesting LvFAS may be involved in WSSV infection [9]. A recent study has shown that in crayfish, WSSV can induce the expression of FAS through the PI3K-Akt-mTOR pathway to improve lipid biosynthesis for supporting viral morphogenesis, indicating FAS could play a positive role in WSSV infection [10]. In this study, we focused our

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attention on the function of LvFAS in infection of *Vibrio parahaemolyticus,* a major bacterial pathogen of shrimp [11]. We demonstrated that LvFAS could play a positive role in immune responses against *V. parahaemolyticus* infection through being involved in regulation of various immune-responsive genes in shrimp. To our knowledge, this is the first report of the antibacterial function of FAS in animals, which could also help us learn more about the shrimp immune system.

2. Materials and methods

2.1. Shrimp immune challenge

The shrimp challenge experiments were performed in a shrimp farm in Zhanjiang City, Guangdong Province, China. Healthy *L. vannamei* (~10 g) were acclimated in air-pumped seawater with a salinity of 2.5% at ~27 °C in 0.8 m^3 tanks and cultured 2 weeks for acclimation before experiments. Five percent of shrimps were randomly detected by PCR to ensure freeing of WSSV, *V. parahaemolyticus* and *V. alginolyticus* as previously described [12,13]. For immune challenge, shrimps were injected with 50 μL PBS containing 2 μg Poly (I:C) or lipopolysaccharide (LPS), or 10^6 colony forming units (CFU) of *V. parahaemolyticus*, which were prepared as previously described [14]. The hemocyte and gill were sampled at 0, 4, 12, 24, 36, 48, 72, 96, and 120 h post injection (hpi), each pooled from 10 shrimps. Shrimps injected with PBS were also sampled as control.

2.2. Real-time PCR

Total RNA were purified using a RNeasy Plus Mini kit (Qiagen, Germany) and reverse transcribed into cDNA using a PrimeScript RT reagent kit (Takara, Japan) according to the manufacturer's instructions. Real-time RT-PCR was performed at a final volume of 10 μL containing 1 μL cDNA, 5 μL 2 × SYBR Premix Ex Taq™ II (Takara, Japan), and 500 nM of each primer on a LightCycle 480 System (Roche, Germany). The optimized thermal cycling parameters were 95 °C for 2 min to activate the polymerase, followed by 40 cycles of 95 °C for 15 s, 62 °C for 15 s and 72 °C for 10 s. Melting curves were generated by increasing the temperature from 72 °C to 95 °C (0.5 °C/s) to denature the double-stranded DNA. The level of LvFAS was determined using $2^{-\Delta\Delta Ct}$ method after normalization to the internal control gene elongation factor 1 alpha (EF1-α, Genbank accession No. GU136229). Sequences of the specific primers were listed in Table 1.

Table 1
Summary of primers used in this study.

Primer	Sequence (5′-3′)
Genome walking	
AP1	GTAATACGACTCACTATAGGGC
AP2	ACTATAGGGCACGCGTGGT
FAS-GWR1	CAGGCATGGTGTCACTTGTG
FAS-GWR2:	CTGTATTCCCCCTTGAGTCGAG
pGL-LvFAS vector construction	
FAS-Pro-AscIF	AATGGCGCGCCAATTATGTGCGACATTATTAAACTCAT
FAS-Pro-FseIR	ATAGGCCGGCCGGAAGACTGAATGCAACGCC
dsRNA synthesis	
dsFAS-T7F	GGATCCTAATACGACTCACTATAGGTCATCACACGAGCCAACATCTG
dsFAS-R	AGCATAGTAAGCCGTAGCATACAC
dsFAS-F	TCATCACACGAGCCAACATCTG
dsFAS-T7R	GGATCCTAATACGACTCACTATAGGAGCATAGTAAGCCGTAGCATACAC
GFP-dsT7F	TAATACGACTCACTATAGGATGGTGAGCAAGGGCGAGGAG
GFP-dsT7R	TAATACGACTCACTATAGGTTACTTGTACAGCTCGTCCATGCC
GFP-dsF	ATGGTGAGCAAGGGCGAGGAG
GFP-dsR	TTACTTGTACAGCTCGTCCATGCC
Real-time PCR	
LvEF1α-F	CCTATGTGCGTGGAGACCTTC
LvEF1α-R	GCCAGATTGATCCTTCTTGTTGAC
LvFAS-F	TTCCATCGCCAGTCTTGTCAG
LvFAS-R	CCACCTTCACCTCGTAGTCAG
ALF1-F	GGATGTGGTGTCCTGGATGG
ALF1-R	GCGTCGTCCTCCGTGATG
ALF4-F	CCTGGTGGCACTCTTCGC
ALF4-R	ACGGTGAAGCGGCACTTATG
CTL2-F	ACAAGCGGAGCAGTTCTGG
CTL2-R	CAGTCACCTTCATAAGACTGATCG
CTL4-F	CTTGGACGCTTATGTCACCTAC
CTL4-R	CATCCTTGCTCTTGATGTAGTCG
CTL5-F	ACGAACCTCCGACAGATGTG
CTL5-R	ACTGGATGACCTGGAAGTGAAG
PEN2-F	CCAAGGCGAAGCGTACAG
PEN2-R	CAATTGCGAGCATCTGAGAC
PEN3-F	CTCCTGCGTCCGCCATG
PEN3-R	GTGTAACCGCCCTTGTACAC
PEN4-F	GCCCGTTACCCAAACCATC
PEN4-R	AACAATCCCCGTATCTGAAGC
Hemolin-F	AGGTCGTCTACAACTGGATCTTCA
Hemolin-R	ACCACGGAGACATCGTTGATC
lysozyme-F	CGGACTACGGCATCTTCCAG
lysozyme-R	TCATCGGACATCAGATCGGAAC
Vpa-16S-F	GGTGTAGCGGTGAAATGCGTAG
Vpa-16S-R	CCACAACCTCCAAGTAGACATCG