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Autophagy genes of *ATG5-ATG12* complex in response to exogenous stimulations in *Litopenaeus vannamei*

Yunhao Yuan, Yongxiong Huang, Junliang luo, Jichang Jian, Shuanghu Cai, Shiping Yang*

Fisheries College of Guangdong Ocean University, Guangdong Provincial Key Laboratory of Aquatic Animal Disease Control and Healthy culture & Key Laboratory of Control for Disease of Aquatic Animals of Guangdong Higher Education Institutes, Zhanjiang, 524088, China

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Abstract

Autophagy plays an important role in resisting pathogens infection and environmental stress. However, there are few studies on autophagy and its regulation in *Litopenaeus vannamei*. In this study, the autophagy-related genes of *ATG5-ATG12* complex (*ATG5*, *ATG7*, *ATG10* and *ATG12*) were cloned and investigated on the response to exogenous stimulations in *L. vannamei*. Multiple sequence alignment and phylogenetic analysis of different species showed that four autophagy genes were conserved among different species. Tissue detection showed that the four autophagy genes were expressed in all tissues, and the expression level was the highest in the hepatopancreas in *L. vannamei*. Furthermore, the expression levels of the four autophagy genes were up-regulated significantly after stimulation with *Vibrio harveyi* and the virus analog *poly(I:C)* ($p<0.05$), and their peak values occurred at 24-48h. These results indicated that *ATG5*, *ATG7*, *ATG10* and *ATG12* may be involved in resisting pathogen infection in *L. vannamei*, which provided a basis for studying the molecular mechanism of autophagy in resistance to pathogen infection of *L. vannamei.*

* Corresponding author. e-mail: ysp20010@sina.com

Introduction

Autophagy is an evolutionarily conserved catabolic process that generally promotes cell survival by eliminating intracellular pathogens by removing damaged organelles (Levine and Kroemer, 2008). In some specific cases, autophagy can also act as a non-apoptotic form of programmed death through excessive self-consumption or selective degradation of growth-promoting factors. Autophagy is controlled by a group of autophagy-related genes (ATGs) that work together during autophagy generation (Berry and Baehrecke, 2007, Denton et al., 2009). The field of autophagy research has been extended to higher animal and plant cells and has achieved fruitful results (Klionsky, 2008). For example, the starvation response induces autophagy to break down macromolecules of the cell's metabolism, producing intermediate metabolites required for catabolic and anabolic processes (Rubinsztein et al. 2011). It has been recognized that more than 30 autophagy-related proteins are involved in the driving of autophagy, and some are well-conserved (Mizushima and Komatsu, 2011, Jordan and Randall, 2012).

During usual circumstances, autophagy serves a wide range of physiological functions and is crucial for preserving the equilibrium of the intracellular environment. Normal proteins are degraded by autophagy as animals age and differentiate (Luo et al., 2009). Autophagy also plays a role in cell death and inhibition of tumorigenesis (Rubinsztein et al., 2011). Autophagy is required for development and is critical for maintaining homeostasis in adult organisms (Mizushima and Levine, 2010). In many physiological and pathological conditions, autophagy is a protective mechanism that promotes the degradation of excess or damaged cellular components for the subsequent recycling of amino acids, lipids, nutrients, and metabolites (Mizushima and Levine, 2010). However, abnormal levels of autophagy can lead to many diseases, such as tumors, diabetes, neurodegenerative diseases, and dilated cardiomyopathy (He et al., 2020, Li et al., 2019). Generally, the *ATG5-ATG12* ubiquitin-like binding system is required to induce conventional autophagy (Mizushima and Komatsu, 2011). During autophagy, the E1 ubiquitin-active enzyme *ATG7* recruits *ATG12* with the E2 ubiquitin-conjugating enzyme *ATG10* to form an *ATG12-ATG10*sulfolipid intermediate, which further binds to *ATG5* to form an *ATG12-ATG5* coupling. Then *ATG5-ATG12* conjugates further mediate the binding of phosphatidylethanolamine (PE) to LC3 to promote autophagy formation (Kaiser et al., 2013). The immune regulation of autophagy has attracted more and more attention in recent years, and studies have successively demonstrated that autophagy can play a role in immune regulation (Martin et al., 2012).

Litopenaeus vannamei is the world's most crucial shrimp culture species and has a high economic value (Liang et al., 2016). However, the frequent occurrence of diseases caused by *Vibrio* infections and virus infections has caused substantial economic losses (Lightner, 2011). The transcriptome sequencing results showed that autophagy increased significantly after *V. alginolyticus* infection (Wang et al., 2022b). Ammonia nitrogen stress also results in an increase in autophagy levels (Wang et al., 2022a). However, the molecular mechanism of autophagy and its role in pathogen infection and environmental stress in *L. vannamei* remains unclear.

The present study mainly revealed possible relationships between autophagy and exogenous stimulation in *L. vannamei.* Based on the traditional autophagic pathway of action, autophagy genes of *ATG5-ATG12* complex were cloned and investigated in response to the stimulations of *V. harveyi* and *poly(I:C)* in *L. vannamei* for understanding the role of autophagy in the process of resistance to exogenous stimulation.

Materials and Methods

Experimental animals

L. vannamei was purchased from local aquaculture farmers. The healthy shrimps with a weight size of about (7.7±0.34) g were selected. Before the test, shrimps were acclimated in the laboratory for 7 days. The water temperature was about 28°C, the salinity was maintained at about 25 ‰, and shrimps were fed three times a day.

Ninety healthy and vigorous shrimp individuals were randomly divided into three groups. Each group was performed in triplicate. Shrimps were infected with *V. harveyi* (1×10^6 CFU/mL), *poly(I:C)* (20 mg/mL) or PBS (as control), respectively. The hepatopancreas, gills, and intestines of *L. vannamei* are susceptible to exogenous stimulations, therefore, they were collected at 0, 6, 12, 24, 48, and 72h after exogenous stimulation and subjected to total RNA extraction. The cDNA templates and RT-PCR were carried out according to the previously mentioned methods.

Total RNA extraction and cDNA synthesis

The hepatopancreas, muscle, and other tissues of *L. vannamei* were sampled. And total RNA was extracted using RNA extraction kit (Trans). Then first-strand cDNA was synthesized using a reverse transcription kit. The synthesized cDNA product was stored at -20°C for subsequent experiments.

Gene cloning and tissue expression analysis

According to the transcriptional sequence, the specific primers for the autophagy gene of *L. vannamei* were designed **(Table 1)**, and the target genes were amplified by PCR using the synthesized cDNA product as a template. The purified product was ligated with pMD-18T (Takara) vector, and transformed into *E. coilDH5α* (Takara) competent cells. The positive clone was sequenced.

The isoelectric points and molecular weights of the four autophagy genes were predicted using the online website (https://web.expasy.org/protparam/). The structures and characteristics of the amino acids encoding the two genes were analyzed using the CDD database of NCBI. The NCBI website (https://blast.ncbi.nlm.nih.gov/) was used to align homologous sequences among multiple species, and MEGA6.0 software was used to construct a neighbor-joining (NJ) evolutionary tree. Bootstrapping was performed using 1000 repetitions to test relative support for a particular clade.

The hepatopancreas, muscle, gill, epidermis, intestine, hemolymph, brain, and ventral nerve cord of healthy *L. vannamei* were collected for tissue expression characteristics analysis by RT-PCR primers were designed according to the obtained sequences of *L. vannamei* **(Table 1)**, and *EF1α* was used as an internal reference. The total reaction volume is 20μL, including 1μL cDNA template, 5μL TB Green® Premix Ex Taq™ II (Takara), 1μL forward primer, 1μL reverse primer, ddH_2O 2μL. The reaction program was: pre-denaturation at 95°C for 5min; denaturation at 95°C for 10s, annealing at 60°C for 20s, extension at 72°C for 20s, 40 cycles.

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Table 1 Primer information required for the test

Primer name	Primer sequence (5' - 3 ')
LvATG5-R	ATGGCTGAAGACAGGGAGATC
LvATG5-F	TCATGACATCTGAGTGGGGATATAA
LvATG7-R	ATGCAGCAATTGCAATC
LvATG7-F	TCACTCCATCTCCTCCTC
LvATG10-R	ATGGGCACCATATCATATGAAGA
LvATG10-F	TTATTGGCACTGGAAATAAAGTATT
LvATG12-R	ATGGAGGGCGAGAAGG
LvATG12-F	TCAACCCCAAGCTTGG
QLvATG5-R	CCCCAGACCCTTACTACCTC
QLvATG5-F	TTCCAACCACATTTCAGCAT
QLvATG7-R	TCCGCCAGAGTCTGTTTGTC
QLvATG7-F	GGAAGCCATCTGCTTTCCCT
QLvATG10-R	GGATGCTCTTGTTGCGTCAGG
QLvATG10-F	CAATCACTCGGGTAAACTTCT
QLvATG12-R	CTTACAATAGTGAAGCACCAGTT
QLvATG12-F	GGAGAAGCCAGTAGGTTGGGTAG
EF1α-R	CCTTTTCTGCGGCCTTGGTAG
EF1α-F	TATGCTCCTTTTGGACGTTTTGC

Analysis

Two-way ANOVA was performed using SPSS11.0 software, and the data obtained from the experiments were statistically and analytically analyzed using Duncan-type multiple comparisons when differences were significant ($P<0.05$ indicates a significant difference).

Results

Gene cloning and sequence analysis

In this study, four autophagy-related genes (*ATG5*, *ATG7*, *ATG10* and *ATG12*) were amplified in *L. vannamei*, and cDNA fragments of the four genes were successfully obtained.

The complete open reading frame (ORF) of the *ATG5* is 810bp, encoding 269 amino acids **(Figure 1)**, the relative molecular weight of the protein is 29.6kDa, and the predicted isoelectric point is 5.59. The results of multiple sequence alignment among different species showed that *ATG5* of different species had more amino acid conservation sites, which also indicates that *ATG5* has better conservation to a certain extent **(Figure 2)**. The results of phylogenetic tree analysis showed that the *ATG5* of *L. vannamei* clustered into a clade with *Penaeus monodon* and *Macrobrachium nipponense*, then with *Blattella germanica* **(Figure 10)**. Compared with other species, *L. vannamei* and *P. monodon* are more closely related. It can also be seen that compared with vertebrates, the *ATG5* gene in *L. vannamei* is closely related to invertebrates.

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   1 M  A  E  D  R  E  I  L  R  E  V  W  D  G  R  V  A  V  C  V  Q  L  A  S  E  D  C  N  T  L
   1 ATGGCTGAAGACAGGGAGATCTTGCGTGAAGTGTGGGATGGTAGAGTCGCAGTTTGCGTTCAGCTTGCGAGTGAAGATTGCAACACGCTG
  31 S  A  P  D  P  Y  Y  L  M  V  P  R  L  S  Y  F  P  L  V  T  D  K  V  R  K  H  F  L  R  F
  91 AGTGCCCCAGACCCTTACTACCTCATGGTTCCAAGACTATCCTACTTCCCTTTAGTTACGGATAAGGTACGAAAACATTTTCTCCGCTTC
  61 I  T  T  E  L  N  D  A  E  M  W  L  E  S  D  G  T  P  L  K  W  H  Y  P  V  G  V  L  F  D
 181 ATCACAACAGAACTCAATGATGCTGAAATGTGGTTGGAATCAGATGGTACTCCTTTGAAATGGCATTATCCCGTTGGTGTTCTGTTTGAC
  91 L  H  C  G  G  A  S  L  P  W  N  L  T  A  H  F  S  H  F  P  E  Q  E  L  I  K  C  S  S  R
 271 CTTCATTGTGGTGGCGCTTCCCTTCCTTGGAACCTCACTGCCCACTTTTCTCACTTCCCAGAGCAAGAGCTCATCAAGTGTTCTTCCAGA
 121 E  V  V  E  S  H  F  L  S  S  L  K  E  A  D  G  L  K  H  R  G  Q  I  I  T  N  M  Q  S  K
 361 GAAGTTGTAGAGTCCCATTTCCTTTCATCCTTAAAAGAAGCTGATGGTTTGAAGCACAGAGGGCAGATCATTACCAATATGCAAAGCAAG
 151 D  H  K  Q  L  W  M  G  L  C  N  D  K  F  D  Q  F  W  A  I  N  R  K  L  M  D  S  T  P  E
 451 GATCACAAGCAATTATGGATGGGCCTTTGTAATGATAAGTTTGATCAGTTTTGGGCCATTAATCGGAAGCTGATGGATAGTACCCCAGAG
 181 E  G  F  K  H  I  P  F  R  L  H  L  P  C  Q  P  P  I  Q  R  L  I  R  P  L  T  D  D  G  R
 541 GAAGGTTTTAAACACATTCCCTTCCGTCTGCACTTACCCTGCCAGCCCCCAATTCAGAGGCTTATCAGACCTCTCACAGATGACGGAAGG
 211 K  V  T  L  G  D  L  L  Q  E  F  L  P  D  S  S  N  E  G  D  R  V  V  I  Q  G  I  E  A  P
 631 AAGGTTACATTGGGTGATCTCCTGCAAGAATTTTTACCCGACTCTAGTAATGAAGGAGACCGCGTAGTGATCCAGGGAATCGAGGCCCCA
 241 R  E  T  P  L  Q  W  L  S  E  H  L  S  H  P  D  N  F  L  H  I  C  Y  I  P  T  Q  M  S  *
 721 CGAGAGACTCCATTACAGTGGCTTAGTGAACATCTTAGTCACCCAGATAATTTTCTTCATATTTGTTATATCCCCACTCAGATGTCATGA
```

Figure 1 *cDNA sequence and deduced amino acid sequence of ATG5*

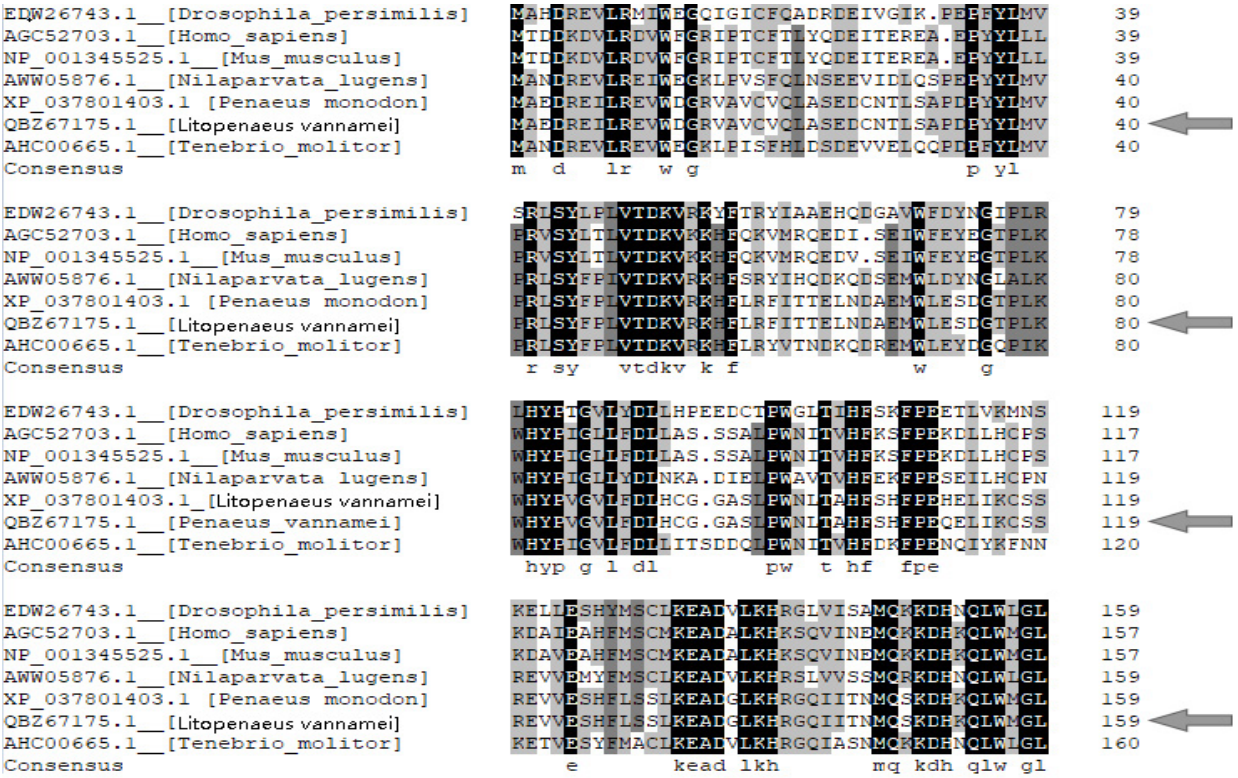

Figure 2 *Multiple sequence alignment of ATG5 from different species Note: Gene accession numbers: Drosophila persimilis, EDW26743.1; Homo sapiens, AGC52703.1; Mus musculus, NP_001345525.1; Nilaparvata lugens, AWW05876.1; Penaeus monodon, XP_037801403.1; Litopenaeus vannamei, QBZ67175.1; Tenebrio molitor, AHC00665.1.* (*Litopenaeus vannamei* marked by arrows).

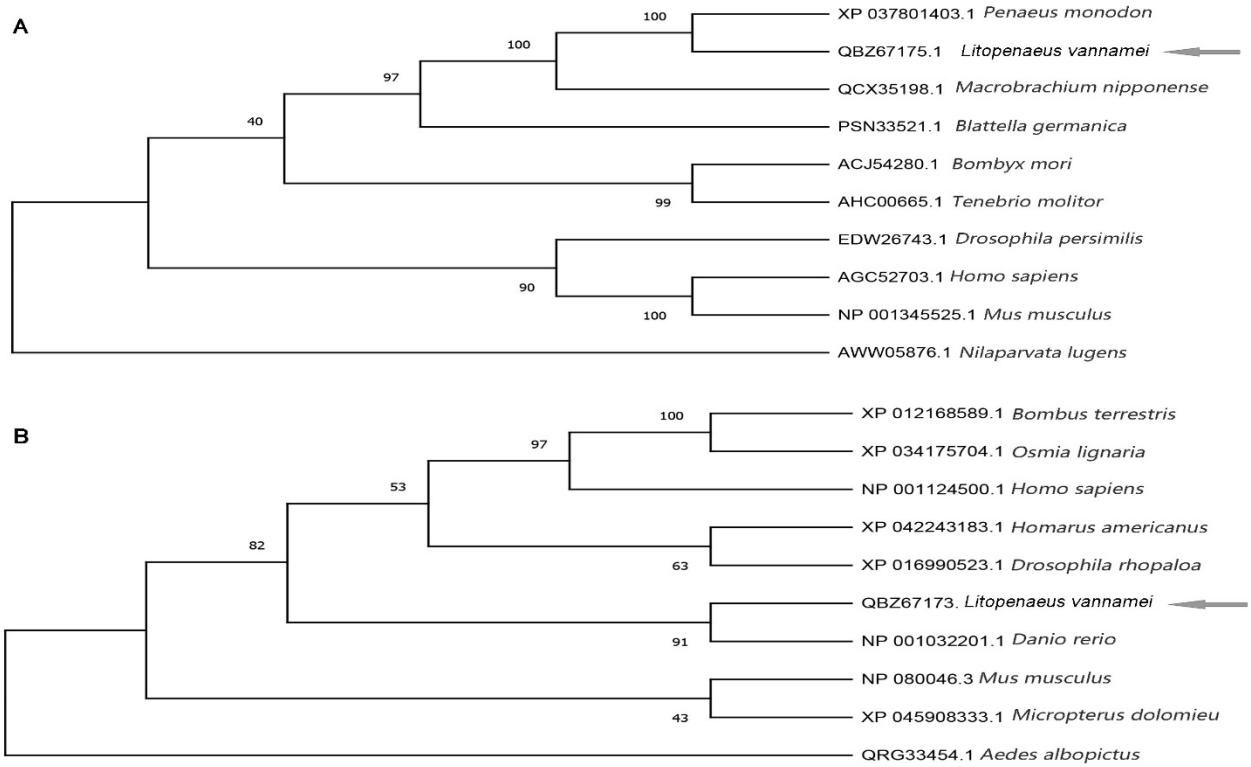

Figure 3 Phylogenetic analysis of amino acid sequences of *ATG5* and *ATG10* from different species (A: *ATG5*; B: *ATG10*). Note: (*ATG5*) Gene accession number: *Macrobrachium nipponense*, QCX 35198.1; *Blattella germanica*, PSN33521.1; *Bombyx mori*, ACJ54280.1; *Drosophila persimilis*, EDW26743.1; *Homo sapiens*, AGC52703.1; *Nilaparvata lugens*, AWW05876.1; *Penaeus monodon*, XP_037801403.1; *Litopenaeus vannamei*, QBZ67175.1; *Tenebrio molitor*, AHC00665.1. (*ATG10*) Gene accession number: *Litopenaeus vannamei*, QBZ67173.1; *Bombus terrestris*, XP_012168589.1; *Homo sapiens*, NP_001124500.1; *Osmia lignaria*, XP_034175704.1; *Mus musculus*, NP_080046.3; *Homarus americanus*, XP_042243183.1; *Danio rerio*, NP_001032201.1; *Drosophila rhopaloa*, XP_016990523.1; *Micropterus dolomieu*, XP_045908333.1; *Aedes albopictus*, QRG33454.1. (*Litopenaeus vannamei* marked by arrows).

The ORF of the *ATG7* was 1212 bp **(Figure 4**), encoding 404 amino acids, with a relative molecular weight of 99.7kDa and a predicted isoelectric point of 5.03. Multiple sequence comparisons between different species showed that the protein encoded by *ATG7* in the *L. vannamei* had a high degree of the results of the phylogenetic tree analysis showed that the *ATG7* protein encoded by *L. vannamei* had high sequence similarity and was well conserved with other species of *Arthropoda* and *Chordata*. **(Figure 5)** The results of the phylogenetic tree analysis showed that *L. vannamei* and *Osmia lignaria* converged into one clade, and to a lesser extent, merged into one clade with the *Bombyx mori* **(Figure 6)**. Compared to mammals, the kinship between the three species mentioned above is significantly higher.

Figure 4 *cDNA sequence and deduced amino acid sequence of ATG7*

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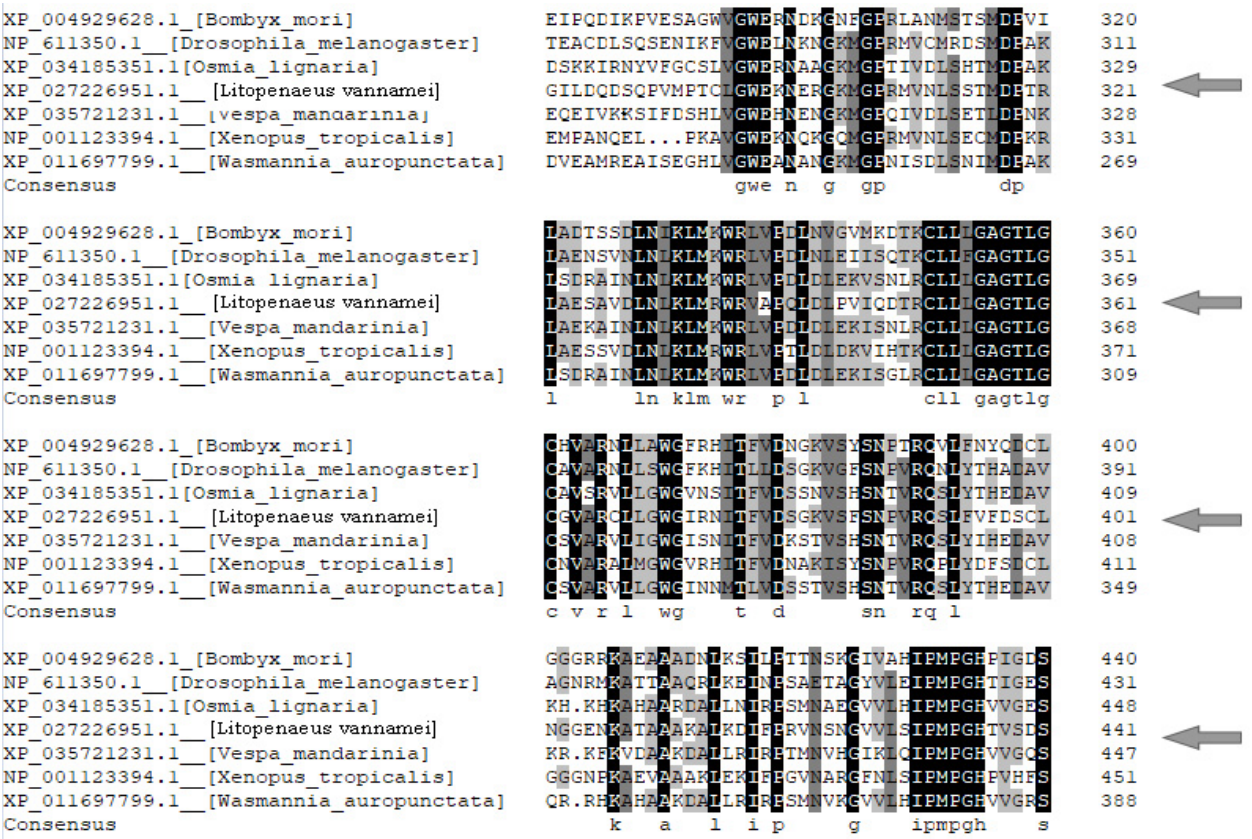

Figure 5 Multiple sequence alignment of *ATG7* from different species. Note: Gene accession number: *Wasmannia auropunctata*, XP 011697799.1; *Litopenaeus vannamei*, XP 027226951.1; *Xenopus tropicalis*, NP001123394. 1, *Bombyx mori*, XP004929628.1; *Osmia lignaria*, XP034185351.1; *Drosophila melanogaster*, NP 611350.1; *Vespa mandarinia*; XP035721231.1. (*Litopenaeus vannamei* marked by arrows)

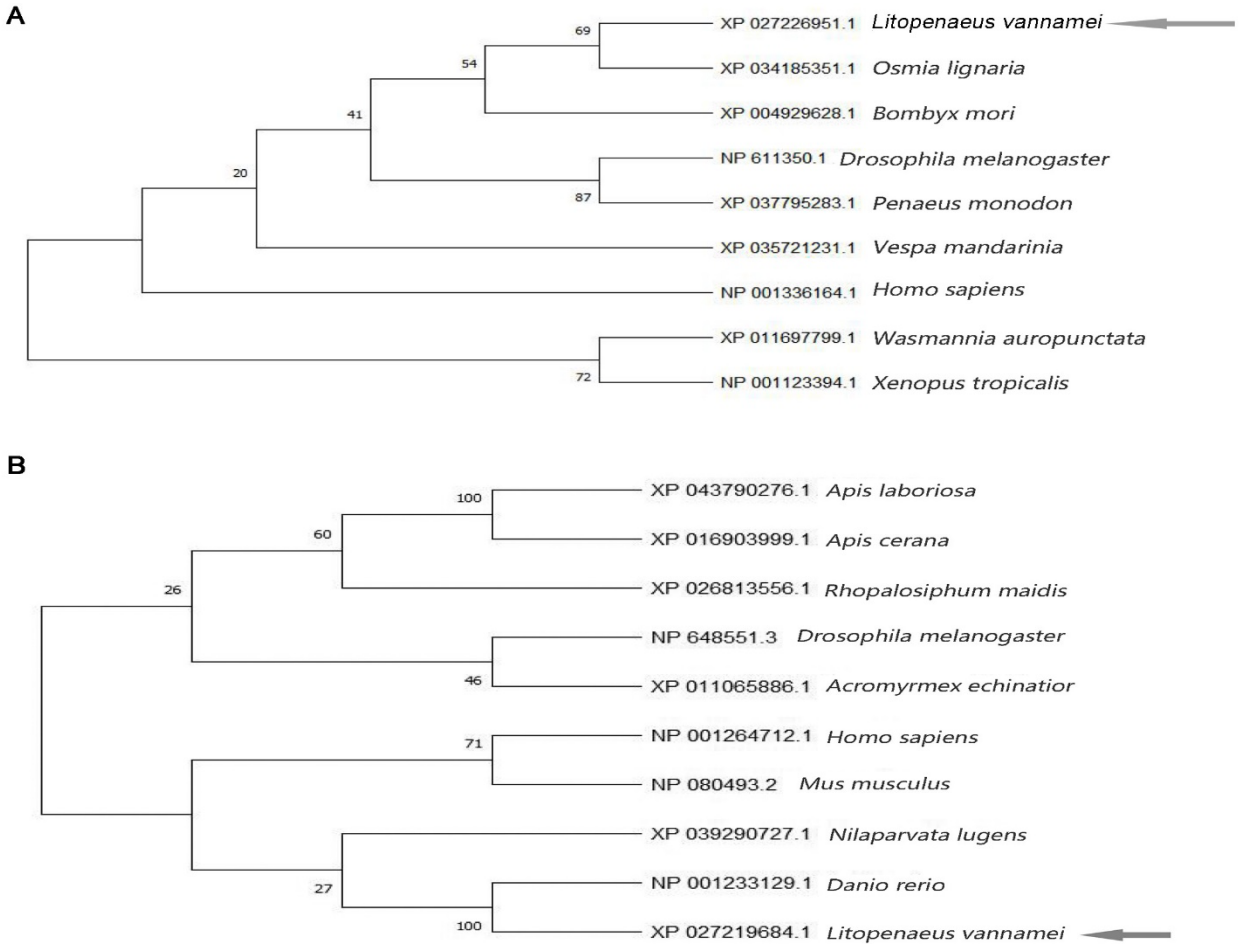

Figure 6 Phylogenetic analysis of amino acid sequences of *ATG7* and *ATG12* in different species (A: *ATG7*; B: *ATG12*). Note：(ATG12) Gene Accession Numbers: *Apis laboriosa*, XP 043790276.1; *Apis cerana*, XP 016903999.1; *Rhopalosiphum maidis*, XP 026813556.1; *Drosophila melanogaster*, NP 648551.3; *Acromyrmex echinatior*, XP 011065886.1; *Homo sapiens*, NP 001264712.1; *Mus musculus*, NP 080493.2; *Nilaparvata lugens*, XP 039290727.1; *Danio rerio*, NP 001233129.1; *Litopenaeus vannamei*, XP 027219684.1. (*ATG7*) Gene Accession Numbers: *Wasmannia auropunctata*, XP 011697799.1; *Litopenaeus vannamei*, XP 027226951.1; *Xenopus tropicalis*, NP001123394.1, *Bombyx mori*, XP 004929628.1; *Osmia lignaria*, XP 034185351.1; *Drosophila melanogaster*, NP 611350.1; *Vespa mandarinia*; XP035721231.1. *Homo sapiens*, NP001336164.1. (*Litopenaeus vannamei* marked by arrows).

The ORF of the *ATG10* is 669 bp, encoding 222 amino acids **(Figure 7)**, and the relative molecular weight of the protein is 25.6kDa, with a predicted isoelectric point of 5.09. The multiple sequence comparison results indicate that there are more amino acid conservation sites for *ATG10* in different species, which also indicates that the *ATG10* gene is to some extent well conserved **(Figure 8)**. The results of phylogenetic tree analysis showed that the ATG10 gene of *L. vannamei* clustered only with *Danio rerio* and was relatively poorly related to other species **(Figure 6)**.

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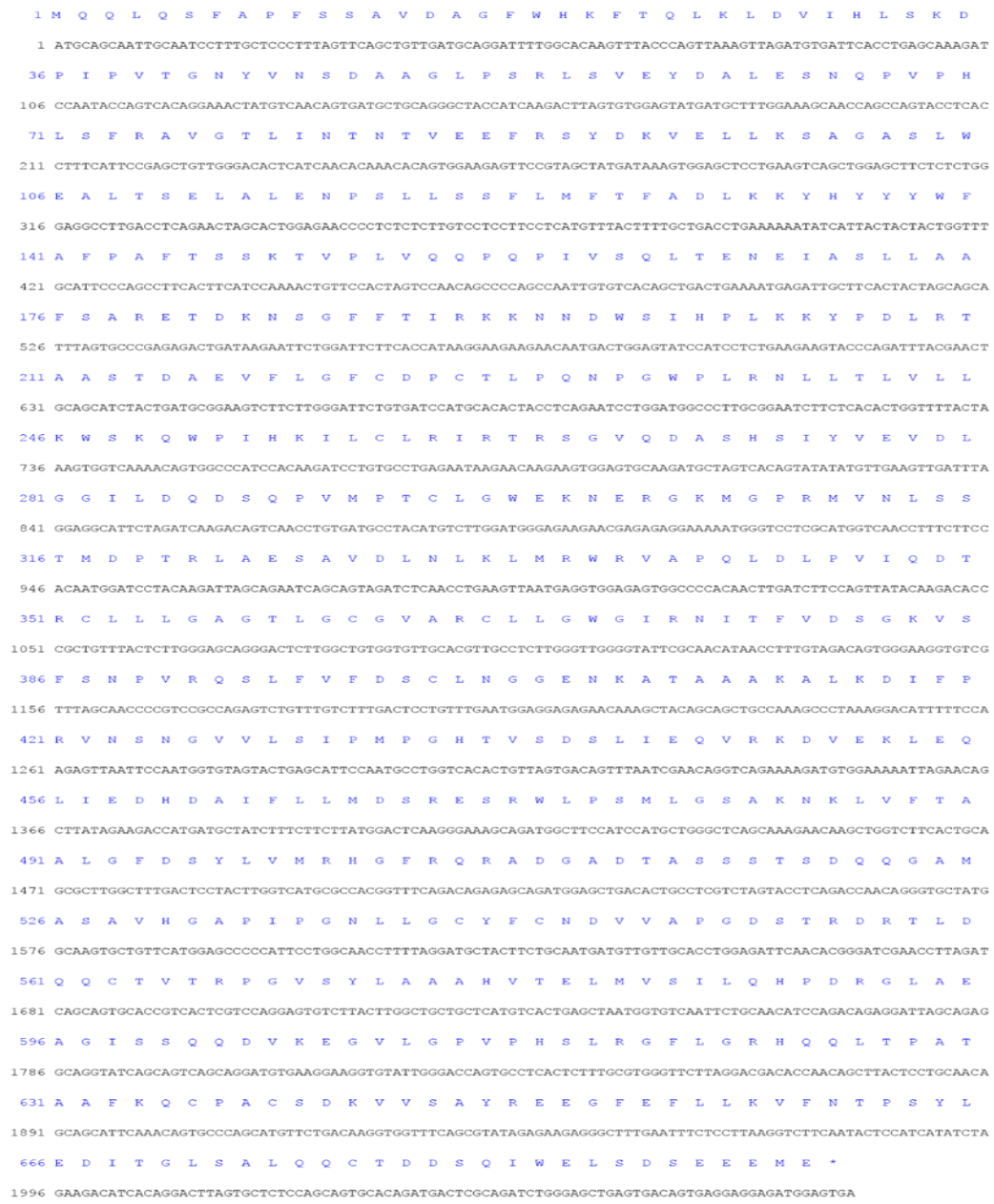

Figure 7 cDNA sequence and deduced amino acid sequence of *ATG10*

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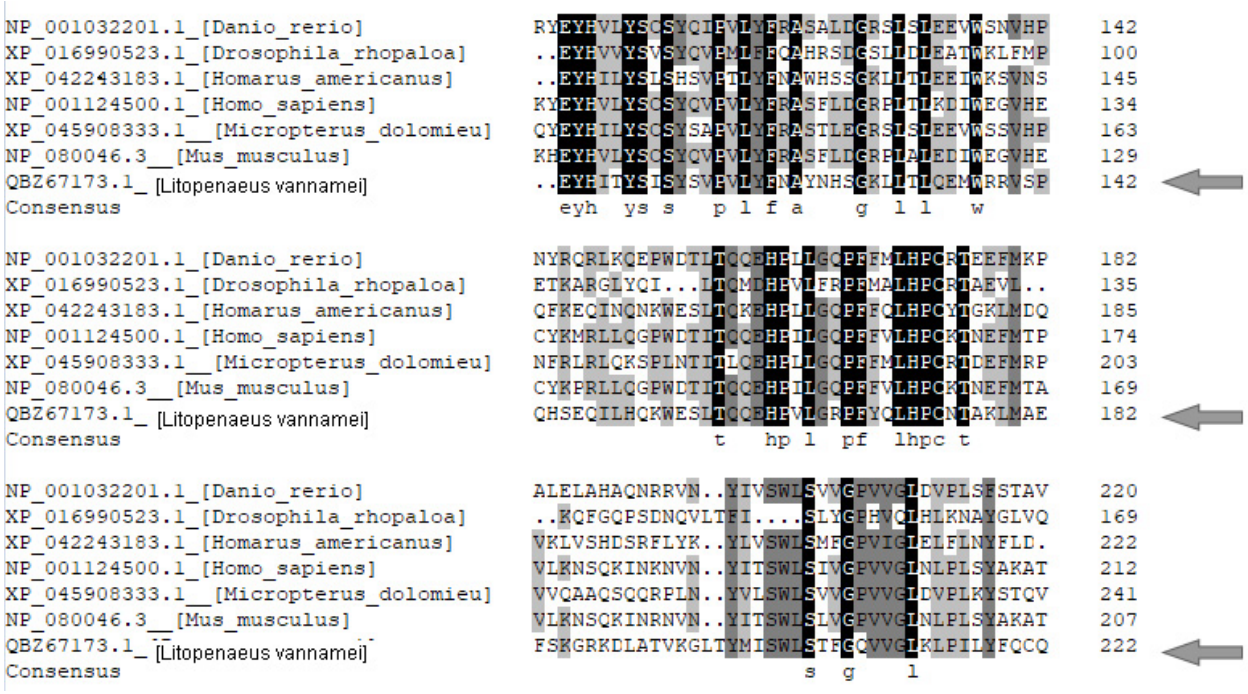

Figure 8 Multiple sequence alignment of *ATG10* from different species. Note: Gene accession number: *Litopenaeus vannamei*, QBZ 67173.1; *Homo sapiens*, NP_001124500.1; *Mus musculus*, NP_080046.3; *Homarus americanus*, XP_042243183.1; *Danio rerio*, NP_001032201.1; *Drosophila rhopaloa*, XP_016990523.1; *Micropterus dolomieu*, XP_045908333.1; *Aedes albopictus*, QRG33454.1. (*Litopenaeus vannamei* marked by arrows).

The ORF of the *ATG12* gene is 363 bp, **(Figure 9)** encodes 121 amino acids, the relative molecular weight of the protein is 29.1kDa, the predicted isoelectric point is 5.27, and a Ubl structural domain is present. The results of multiple sequence comparisons showed that the *ATG12* sequence of *L. vannamei* differed significantly from those of *Mus musculus* and *Homo sapiens*. In contrast, the gene sequence similarity with species such as *Apis laboriosa* and *A. cerana* was higher **(Figure 10)**, indicating that *ATG12* is better conserved in invertebrates. The phylogenetic tree of the analysis showed that *L. vannamei* had the highest affinity with the white shrimp and *Nilaparvata lugens* **(Figure 3)**.

Figure 9 cDNA sequence and deduced amino acid sequence of *ATG12* (marked in red as Ubl conserved domains)

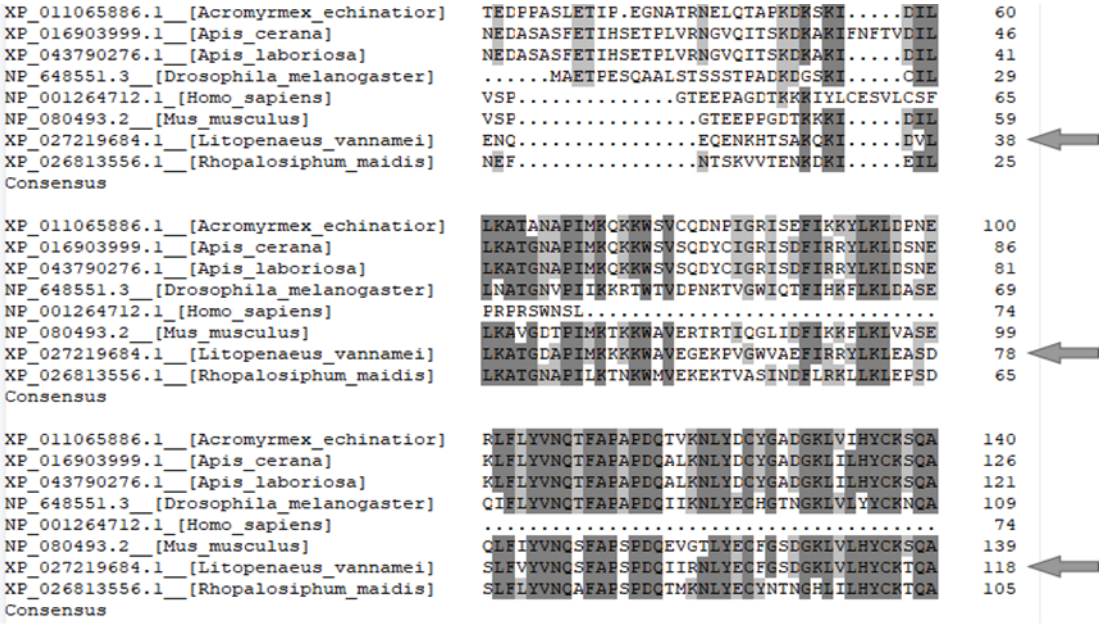

Figure 10 Multiple sequence alignment of *ATG12* from different species.Note: Gene Accession Numbers: *Apis laboriosa*, XP 043790276.1; *Apis cerana*, XP 016903999.1; *Rhopalosiphum maidis*, XP 026813556.1; *Drosophila melanogaster*, NP 648551.3; *Acromyrmex echinatior*, XP 011065886.1; *Homo sapiens*, NP 001264712.1; *Mus musculus*, NP 080493.2; *Litopenaeus vannamei*, XP 027219684.1. (*Litopenaeus vannamei* marked by arrows)

Expression characteristics of tissue distribution

The expressions of *ATG5*, *ATG7*, *ATG10*, and *ATG12* in different tissues of *L. vannamei* were detected by RT-PCR. The specific results were shown in **Figure 11**. Four autophagy-related genes were expressed in all eight tissues of *L. vannamei*, and the expression profiles were similar to a certain extent. The results showed that the expression of the four autophagy

 https://doi.org/10.46989/001c.55792

genes in the hepatopancreas was the highest, which was about 10-fold higher than those in the tissues with the lowest expression levels. This indicated that autophagy occurs mainly in the hepatopancreas of *L. vannamei*.

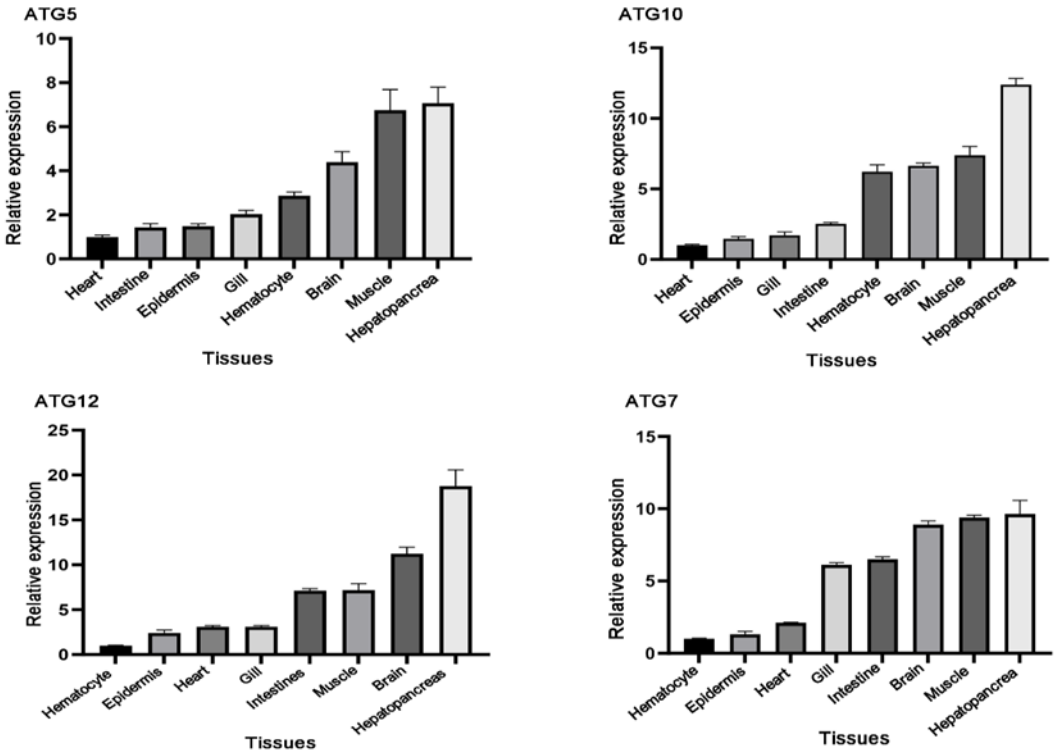

Figure 11 qPCR detection of the four autophagy genes mRNA expression patterns in different tissues of healthy shrimp (All values are mean ± SD; n= 3).

Expression profiles of the four autophagy genes after exogenous stimulation

In the *poly(I:C)* and *V. harveyi* groups, the expressions of autophagy genes *ATG5*, *ATG7*, *ATG10*, and *ATG12* in hepatopancreas, intestinal tract, and gill tissues were significantly changed compared with those in the PBS group ($P<0.05$) **(Figure 12)**. The stimulation of *poly(I:C)* and *V. harveyi* significantly increased the expression level of autophagy genes. The expression level of four autophagy genes showed a peak of 12h-24h after infection, and then the expression level decreased significantly. In the two experimental groups, the expression levels of the four autophagy genes investigated in the hepatopancreas were higher than those in the other two tissues. The expression levels changed more significantly with the delay of infection.

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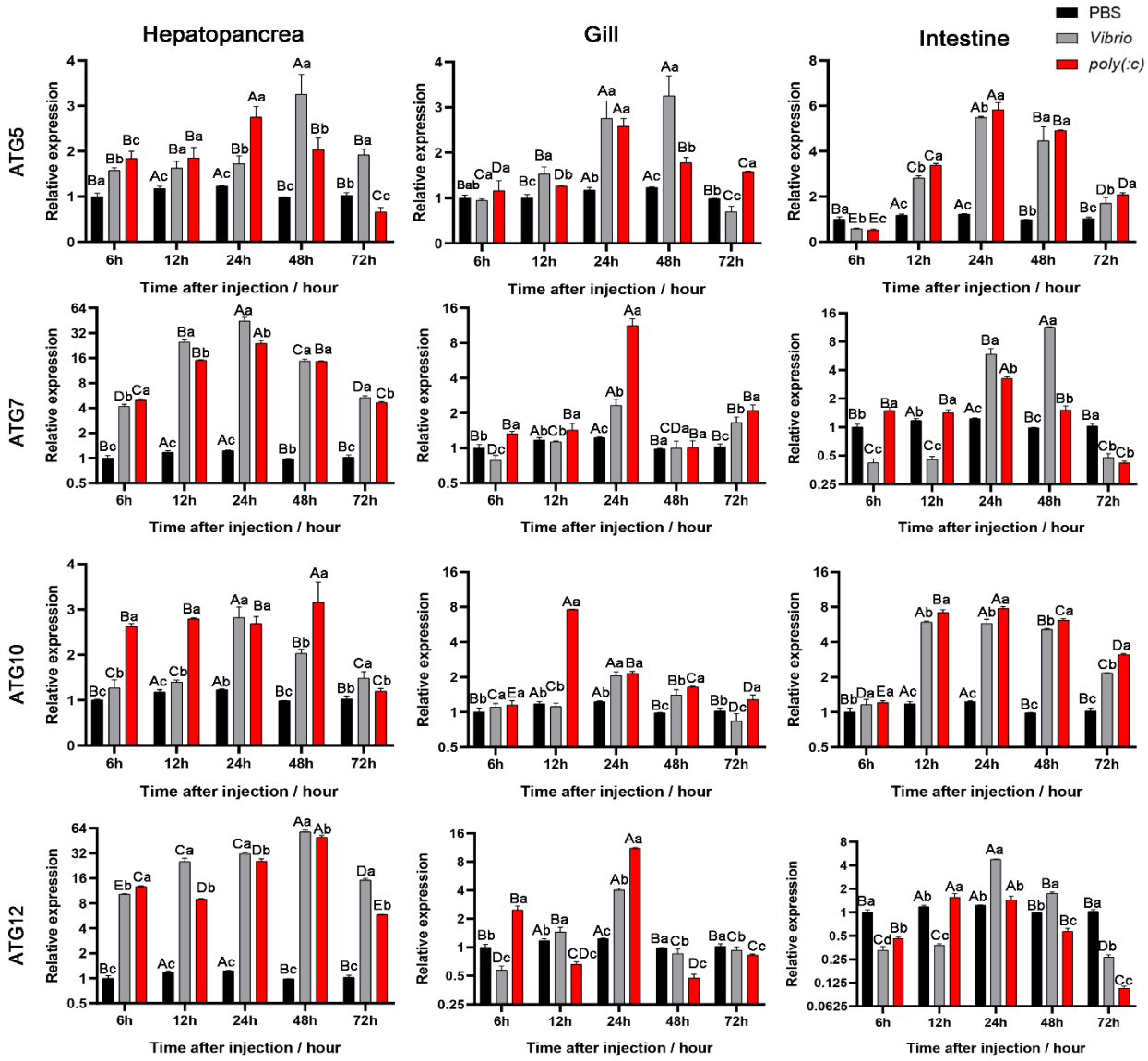

Figure 12 Expression changes of the four autophagy genes in different tissues under *Vibrio harveyi* and *poly(I:C)* stimulation. All values are mean ± SD; n= 3. The expression level of PBS group was set to 1.00. $p<0.05$ indicates a significant difference. Lowercase letters indicate significant differences between stimuli at the same time, and uppercase letters indicate significant differences between stimuli at the same time.

Discussion

Autophagy, as the "Housekeeping" of the cell, is controlled by autophagy-related genes (ATGs) and other factors, and its main role is to maintain the homeostasis of the intracellular environment by eliminating unwanted or harmful substrates, a process that is indispensable for maintaining the cellular victory homeostasis (Wei et al., 2021, Fader and Colombo, 2009). In mammals, *ATG12* plays an important role in the formation of autophagosome membranes by coupling with *ATG5* to form *ATG12-ATG5* conjugates (Kuma et al., 2002), while *ATG7* activates and binds *ATG12* in an ATP-dependent manner and subsequently forms a sulfolipid intermediate with *ATG10* to further mediate autophagy (Mizushima, 2020). Several studies

have shown that autophagy and autophagy-related genes are involved in the immune response to viral and *Vibrio* infections (Jackson, 2015; Jounai et al., 2007). However, many autophagy-related genes have not been studied in *L. vannamei*. In this study, we cloned and identified four autophagy-related genes (*ATG5*, *ATG7*, *ATG10* and *ATG12*) from *L. vannamei*. And multiple sequence analysis showed high homology with other species, demonstrating that autophagy is highly conserved *in vivo* (Shibutani et al., 2015).

To study the functional properties of the four autophagy genes, we analyzed the expression profiles of *ATG5*, *ATG7*, *ATG10,* and *ATG12* in the tissues of *L. vannamei*. The results showed that while comparing different tissues, it was found that even though the four autophagy genes were expressed in the tested tissues, the relative expression levels of genes in different tissues were different. The hepatopancreas is the central organ of the digestive and immune system of shrimp (Zhao et al., 2017); it was evident that gene expression levels were higher in the hepatopancreas than in other tissues. In the exogenous stimuli experimental, the changes of expression levels of the autophagy genes in hepatopancreas were more pronounced with the delay of infection time. The results demonstrate that in *L. vannamei*, autophagy is exercised mainly in the hepatopancreas.

In invertebrates, bacterial and viral infections can disrupt homeostasis, leading to tissue damage and even death (Tello-Olea et al., 2019). It has been shown that autophagy will target intracellular pathogens for destruction during innate immunity and will inhibit viral infestation of the organism during *in vivo* immunization (Schmid and Münz, 2007). For example, Streptococci proliferate in *ATG5*-deficient mouse embryonic fibroblasts, whereas in wild-type cells, the bacteria are encapsulated and destroyed by autophagosomes (Nakagawa et al., 2004). Autophagy also inhibits the neurotoxicity of HSV-1 virus in mice. (Orvedahl et al., 2007).

To further explore the expression pattern under exogenous stimuli, we performed infection experiments on *L. vannamei* with *V. harveyi* and the virus mimic *poly(I:C)*. The results showed that the expression of autophagy gene in the experimental group was higher than that in the control group at the beginning of exogenous infection, Stimulation of *V. harveyi* and *poly(I:C)* significantly increased the expression of autophagic genes, and there is a tendency to decrease with increasing duration of infection, a situation also seen in studies on *Crassostrea gigas*(Han et al. 2019, Leng et al. 2021). There is increasing evidence that viruses have evolved to use autophagy to facilitate their infection and replication in a prolonged battle with the immune system (Desai et al., 2015). At the same time, we also found in this experimental work that the expression activation time and the expression peak time of *ATG7* and *ATG10* are often earlier than that of *ATG5* and *ATG12.* To a certain extent, they have an impact on their expression. This is precisely in line with the molecular role in the conventional autophagy pathway (Mizushima, 2020).

In conclusion, the autophagy-related genes of *L. vannamei were* cloned and analyzed. We report that the four autophagy genes were expressed in all tissues. The expression levels of the four autophagy genes were up-regulated significantly after stimulation with *V. harveyi* and *poly(I:C)*, and they can be affected by the time of infection to varying degrees. These results indicated that autophagy genes play a role in response to exogenous stimuli and provided new insights for further exploration of strategies to cope with exogenous stimuli.

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