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RESEARCH REPORT

Molecular characterization of the dual oxidase (*Lv*Duox) gene from the pacific white shrimp *Litopenaeus vannamei*

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Abstract

The reactive oxygen species（ROS） generated by dual oxidases (Duox) play a role in innate immunity in many organisms. In this study, a 4,735 bp full-length cDNA of the Pacific white shrimp dual oxidase (*Lv*Duox) gene was cloned; the sequence included an open reading frame of 4,497 bp, encoding a protein of 1,498 aa with a calculated mass of 173 kDa. Structural analysis revealed that *Lv*Duox contains several domains. Homology analysis revealed that LvDuox exhibits 96.1%, 67.3% and 67.3% sequence identity with *Marsupenaeus japonicas*, *Drosophila melanogaster* and *Acyrthosiphon pisum* Duox, respectively. The mRNA transcripts of *Lv*Duox were detected in all tested tissues. The mRNA expression of *Lv*Duox was significantly induced in the midgut after *Vibrio parahaemolyticus E1* (VPE1) stimulation. After the level of H_2O_2 in the midgut increased, expression of the superoxide dismutase and catalase genes in the midgut increased significantly. These results suggested that the *Lv*Duox gene was upregulated in the midgut after the challenge by VPE1, and antioxidant genes were involved in the regulation of ROS in the shrimp midgut. *Lv*Duox may therefore be a new target for intestinal disease research in the Pacific white shrimp.

Key Words: *Litopenaeus vannamei*; Duox; antioxidant gene; innate immunity

Introduction

In recent years, owing to water pollution and overuse of antibiotics, the intestines of aquatic animals have been exposed to substantial threats and challenges. In the Pacific white shrimp *Litopenaeus vannamei*, the gut is surrounded by various types of bacteria because of its open anatomical structure. Some pathogens and viruses induce inflammation in the mucosa (Qi *et al.*, 2017), thus resulting in damage to the guts of shrimps. *Vibrio Harveyi*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus E1* (VPE1) are common pathogenic bacteria affecting the production of farmed prawns (Martin *et al.*, 2004; Qi *et al.*, 2017), and they pose a great threat to shrimp health. After the balance of intestinal flora is altered, the immune and digestive systems of shrimp may be affected (Artis, 2008; Miyake *et al.*, 2014; You *et al.*, 2014). Therefore, it is crucial to maintain gut-microbiota homeostasis (van Baarlen *et al.*, 2013; Meng *et al.*, 2018) and determine the mechanism of defense against invading pathogens.

The reactive oxygen species (ROS), which are produced by dual oxidase (Duox), have been suggested to be involved in inhibiting pathogenic bacterial infection in the gut (Ha *et al.*, 2005; Yang *et al.*, 2016). ROS include oxygen radicals and some oxidizing agents formed by the partial reduction of oxygen, such as superoxide (O_2^-), hydroxyl (OH^-), ozone (O_3) and superoxide-derived hydrogen peroxide (H_2O_2) (Juven *et al.*, 1996; Skulachev, 1998). They can damage the structure of DNA and the membrane system in eukaryotic cells, and induce lipid peroxidation (Wang *et al.*, 2009). Therefore, ROS are considered to be a major cause of damage in organisms. Five homologs of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox1, Nox2, Nox3, Nox4 and Nox5) and two homologs of dual oxidase (Duox1 and Duox2) can produce ROS (Morand *et al.*, 2009). Duox, a transmembrane protein in host cells, is the

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main enzyme that generates ROS in epithelial cells of many organs (Flores *et al.*, 2010; Kim *et al.*, 2014). The structural organization of Duox is highly conserved in all studied organisms (Kim *et al.*, 2014). Duox comprises a transmembrane domain, a calcium-modulated EF hand domain, a NADPH oxidase domain producing H_2O_2 and an extracellular peroxidase homology domain that converts H_2O_2 into HOCl (Sumimoto, 2008). H_2O_2 and chloride are important components of host gut immunity. In a study of Duox knockdown in flies, Duox has been found to be responsible for host resistance to gut infection in the gut epithelia (Biteau *et al.*, 2008; Buchon *et al.*, 2009; Kim *et al.*, 2014). In *Drosophila*, H_2O_2 participates in the regulation of intestinal epithelial cell renewal by activating intestinal stem cell proliferation (Abid *et al.*, 2000). In *Anopheles gambiae*, Duox-dependent H_2O_2 is involved in gut permeability by forming a dityrosine network at the peritrophic membrane (Kumar *et al.*, 2010). In zebrafish, Duox-produced H_2O_2 facilitates wound healing and has an antimicrobial function (Niethammer *et al.*, 2009; Flores *et al.*, 2010). In addition, in Pacific white shrimp, H_2O_2 is produced and has a role in anti-microbial activity after pathogens enter the hemolymph (Munoz *et al.*, 2000; Gomez-Anduro *et al.*, 2006). Because the Pacific white shrimp is a crustacean that relies on its innate immune system, cloning the Duox gene has important implications for studies investigating resistance to pathogen invasion. However, the existence of Duox gene in the Pacific white shrimp had not been verified, and the mechanism of Duox gene expression in the Pacific white shrimp was unclear.

In the present study, we cloned the full-length cDNA encoding the Duox gene from *L. vannamei*, which we designated *Lv*Duox. Additionally, we investigated the expression of the Duox gene and two antioxidant enzymes (superoxide dismutase and catalase) genes in the gut of *L. vannamei* after infection by VPE1. Moreover, we detected H_2O_2 levels at different times after VPE1 challenge to determine the role of *Lv*Duox in the natural immune defense mechanisms in the gut of *L. vannamei*. The results may provide a new therapeutic target for intestinal diseases of *L. vannamei*.

Materials and methods

Animals and Vibrio parahaemolyticus *E1 challenge*

Adult *Litopenaeus vannamei* (average weight 11±0.12 g) was obtained from Ruizi Seafood Development Co. Ltd. (Qingdao, China). Before the experiment, the shrimp were randomly allocated to six tanks (each containing 50 L seawater), including three control tanks (C1, C2 and C3) for the gene cloning, and three treatment tanks (V1, V2 and V3) for VPE1 challenge, with forty prawns in each tank, and the shrimps were acclimatized at 28±1 °C in aerated and filtered seawater (salinity 30‰) for a week and fed commercial pellets (supplied by Da Le Co. Ltd, Yantai, China). During challenge, VPE1 was added into each treatment tank at a final concentration of 5×10^6 cfu/mL. The VPE1 strain was donated by Dr. Zhaolan Mo from Yellow Sea Fisheries Research Institute Chinese Academy of Fishery Sciences.

RNA extraction and cDNA preparation

Total RNA was extracted using a MiniBEST Universal RNA Extraction kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. RNA degradation and contamination were monitored on 1% agarose gels. The RNA purity and concentration of each sample were checked using a NanoPhotometer® spectrophotometer (Implen, Munich, Germany). The cDNA synthesis was carried out by TransScript II One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, China) in accordance with the manufacturer's instructions, then stored at -20 °C and used as the temple for cloning and PCR analysis.

Cloning and sequencing

The template of cloning was the cDNA of gut from the healthy shrimp. The partial sequence of *Lv*Duox cDNA was obtained from the transcriptome database of our laboratory. The primers were designed based on this partial sequence by Primer Premier5.0 (Table 1). Two pairs of gene-specific primers, D501/502R and D301/302F, were used to clone the 5' end and 3' end of cDNA of *Lv*Duox by the rapid amplification of cDNA ends (RACE) technique according to the standard procedures. The other primers for cloning were designed based on the results of 5' end and 3' end, and were used by cloning the full-length cDNA of the *Lv*Duox by PCR with the PrimeSTAR® GXL Premix (Takara Bio, Inc., Japan). The PCR products were cloned into the *pEASY*®-T1 Simple Cloning Vector (TransGen Biotech, China) and transformed into the Trans5α Chemically Competent Cell (TransGen Biotech, China), the positive recombinants were identified via anti-ampicillin selection. A sequence analysis was performed using a CEQ 8000 Automated Sequencer (Beckman Coulter, Inc., USA).

The sequence similarity of cDNA was analyzed using FASTA and the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI). The theoretical isoelectric point and molecular mass were calculated using the ExPASy Proteomics Server (http://web.expasy.org/compute_pi). The structural domains of the white shrimp *Lv*Duox were predicted using the simple Modular Architecture Research Tool (SMART; Version 7.0) (http://smart.emblheidelberg.de/). Using the MEGA 7.0 software package, a Neighbor-Joining (NJ) phylogenic tree was constructed using the full-length amino acid sequences of some published Duox proteins downloaded from NCBI, and multiple sequence alignments of some Duox proteins from NCBI using the BioEdit software package.

Detecting the level of H_2O_2

To detect the level of H_2O_2 in the midgut of shrimp at different hours after the VPE1 challenge, three midguts were obtained from shrimps at 0 h, 3 h, 6 h, 12 h, 24 h and 36 h during the VPE1 stimulation. Then, the samples were measured with a Hydrogen Peroxide assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

Table 1 Primers used for the Pacific white shrimp *Lv*Duox analysis in the paper

Primers	Sequence(5'-3')
Primers for 5'RACE	
D501R	GTTGTTGTACCAGCCATCGT
D502R	GCCGAGCACAATCCATCTG
Primers for 3'RACE	
D301F	CATCTTCATCTTCGCGCACC
D302F	ATCTGGTCTTCGGAACGTCG
Universal primers for RACE	
NUP	AAGCAGTGGTATCAACGCAGAGT
UPML	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT
UPMS	CTAATACGACTCACTATAGGGC
3'CDS	AAGCAGTGGTATCAACGCAGAGTAC(T)30V N
5-AP-DG	AAGCAGTGGTATCAACGCAGAGTGGGGGGGGGGHN
Primers of cloning vector	
M13F	TGTAAAACGACGGCCAGT
M13R	CAGGAAACAGCTATGACC
Primers for cloning the rest of sequence	
D503F	TCAGATGGATTGTGCTCGGC
D504F	CAGATGGATTGTGCTCGG
D505F	TTATTTCCAGGGCTCTGAAGTGACG
D506F	AGAACTTCCGCAGGAGGCATTT
D303R	CACTTCAGAGCCCTGGAAATAATCA
D304R	AAGAGCCAGTAGCCCACGGT
D305R	AGATTTCCTGCGTCAGACACCT
Primers for qRT-PCR analysis	
LvD1F	ATCAGATGGATTGTGCTCGGC
LvD1R	GACTCAACGGAGCCCCAAGA
SOD1F	TGACGAGAGCTTTGGATCATTCC
SOD1R	TGATTTGCAAGGGATCCTGGTT
CAT1F	GGCTATGGTTCTCGTACTTCCAAGC
CAT1R	GCATTGTATAGGTCCCTTGTTGCA
β-actin1F	GCCCATCTACGAGGGATA
β-actin1R	GGTGGTCGTGAAGGTGTAA

Gene expression by qRT-PCR

To analyze the expression of *Lv*Duox in various tissues of the white shrimp, including heart, hepatopancreas, intestine, eyestalk, gills and proventriculaus, these tissues were obtained from three healthy white shrimps. To analyze the expression of *Lv*Duox, SOD and CAT of the midgut after it affected the VPE1, the midguts of shrimp (six shrimp per group) were extracted at 0 h, 3 h, 6 h, 12 h, 24 h and 36 h after the VPE1 challenge. The genetic expression of all the temples from these tissues was determined by quantitative real-time PCR (qRT-PCR).

All the temples were carried out in triplicate with a total volume of 20 μL containing 10 μL 2×TransStart® Top Green qPCR SuperMix, 2 μL of cDNA, 0.4 μL each of the forward and reverse primers (final concentration 0.2 μM) and 7.2 μL of ddH_2O. The qRT-PCR using SYBR green I dye (TransGen Biotech, China) was performed using the following cycling conditions: denaturation for 30 s at 94 °C, followed by 40 cycles of 5 s at 94 °C, and 30 s at 60 °C. The gene expression analysis primers of *Lv*Duox, CAT and SOD are listed in Table 1.

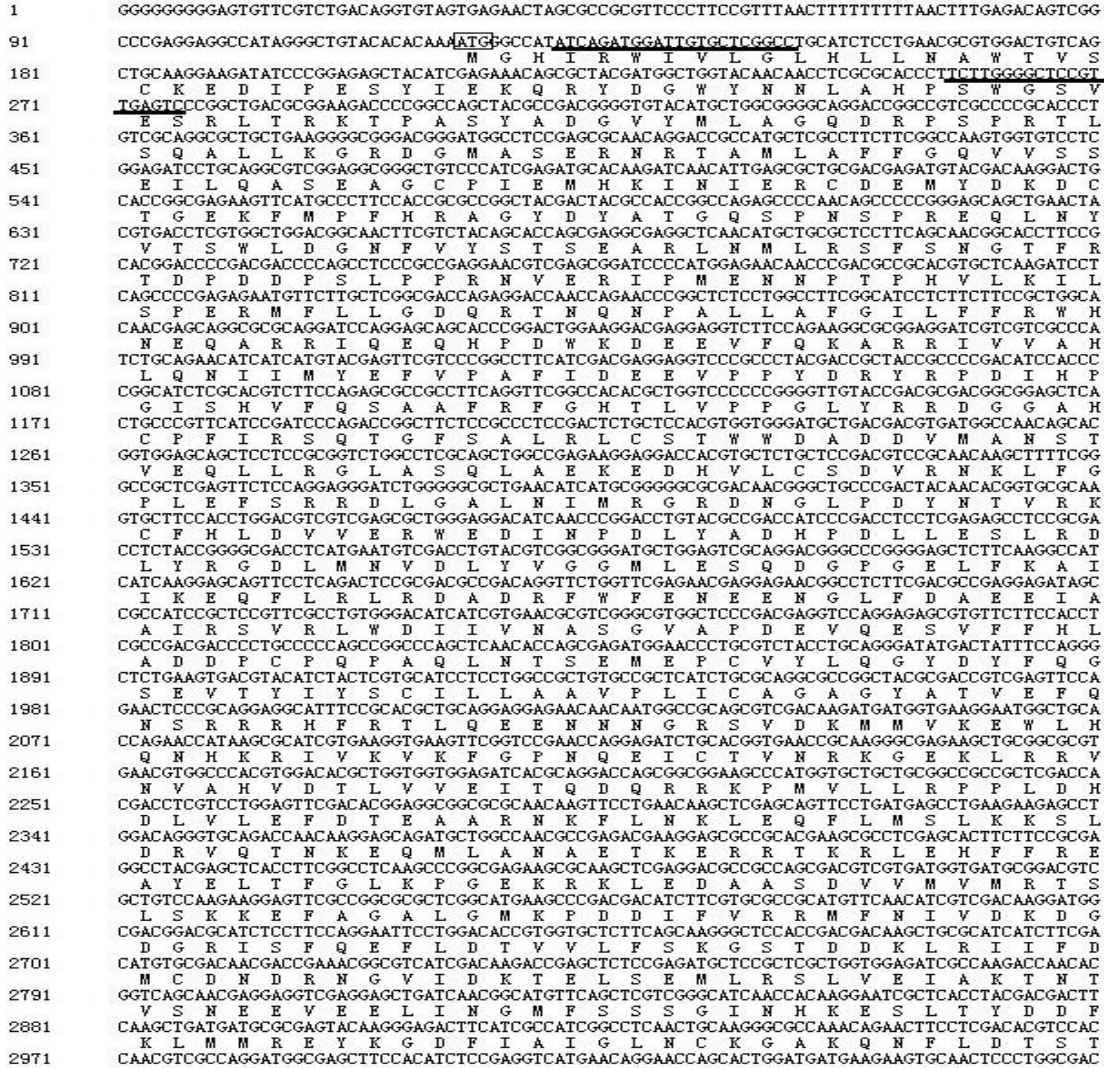

Fig. 1 The nucleotide and deduced amino acid sequences of the *L.vannamei* dual oxidase (*Lv*Duox) cDNA. The sequence has been deposited in the GenBank (accession number MG734366). The cDNA (4735 bp) contains a complete ORF encoding a protein of 1,498 amino acid residues (residue number indicated on the left). The start codon ATG and the stop codon TAA are indicated by the rectangle. The Primer sequences for the qRT-PCR analysis are indicated by the solid lines

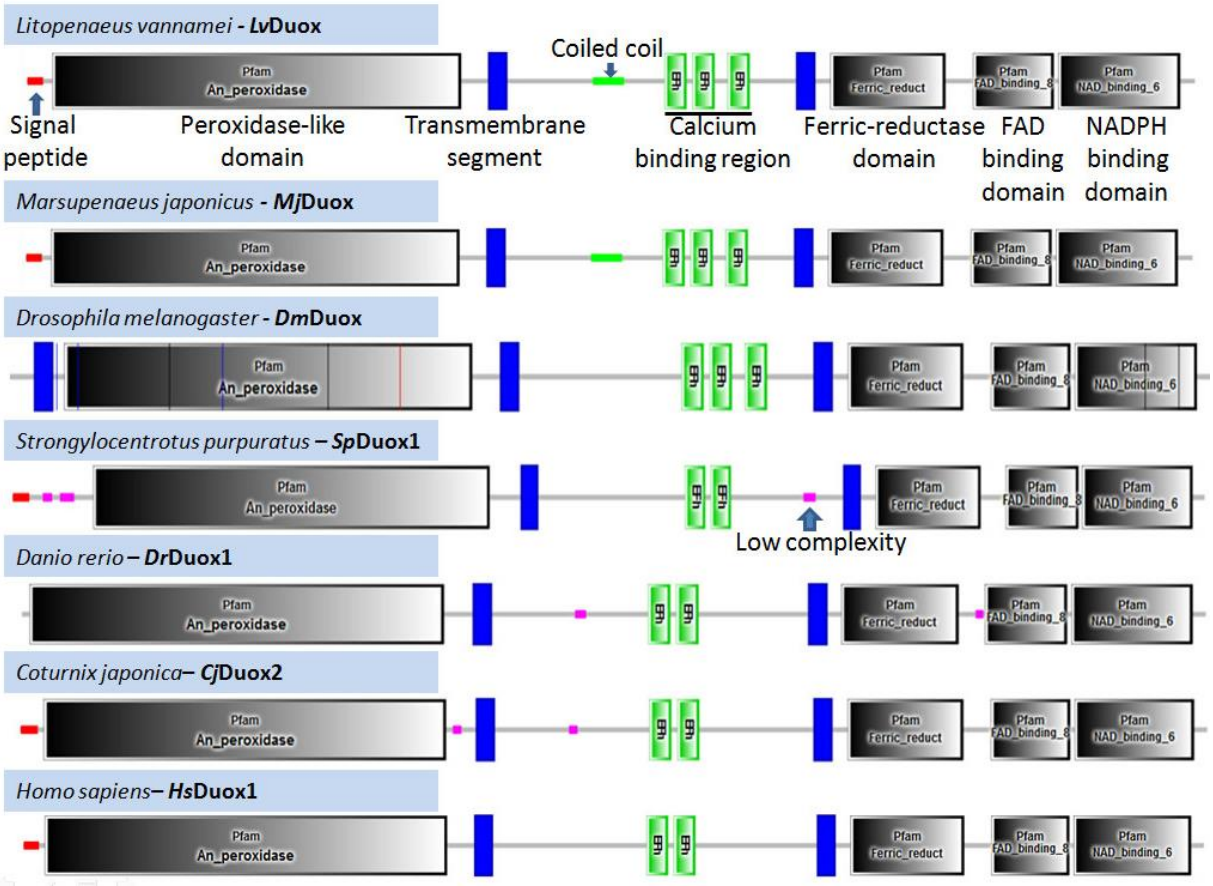

Fig. 2 Comparison of the predicted domain structures of dual oxidases from different organisms. The names of the different domains are marked. The full name, abbreviation and accession number of different Duoxs are listed in the Table 3

Statistical analysis

The expression of *β-actin* gene was used as the reference gene of all the samples, and the comparative CT method ($2^{-\Delta\Delta Ct}$) was used to analyze the expression level of *Lv*Duox and the other genes. The results are expressed as means ± standard deviation (SD). To compare the differences between the data of different groups in different hours, the statistical analysis of these data was performed by one-way analysis of variance (one-way ANOVA) using SPSS Statistics 24.0 software. The $P < 0.05$ was considered statistically significant.

Results

Sequence and domain structures of Lv*Duox*

A 4,735 bp nucleotide sequence of *Lv*Duox was assembled and included an open reading frame (ORF) of 4,497 bp, encoding a protein of 1,498 aa with a calculated molecular mass of approximately 173 kDa and a theoretical isoelectric point of 6.98 (Fig. 1). The cDNA sequence of *Lv*Duox has been deposited in GenBank under accession number MG734366.

To determine the similarity of the complete domain structure of *Lv*Duox to those of other Duoxs, we predicted the structural domains of the Duoxs from different animals by using the Simple Modular Architecture Research Tool (Fig. 2). The deduced amino acid sequence of *Lv*Duox contains a signal peptide (1–21 aa), a peroxidase-like domain (33-557 aa), two transmembrane regions (593–615 aa and 988–1,010 aa), a coiled coil (726–766 aa), three EF-hand motifs (calcium binding region: 818–846 aa, 854–882 aa and 899–927 aa), a ferric reduction region (1,031–1,178 aa), a FAD binding domain (1,214–1,317 aa) and a NADPH binding domain (1,323–1,479 aa).

The structural domains of *Lv*Duox were nearly the same as those of *Mj*Duox, and the peroxidase-like domain, transmembrane segment, ferric-reductase domain, FAD binding domain and NADPH binding domain were conserved among *Lv*Duox and the other Duox proteins. A coiled coil was found only in *Lv*Duox and *Mj*Duox. Moreover, the signal peptide was not present in *Dm*Duox and *Dr*Duox1. As for the calcium binding region, three arthropod Duoxs (*Lv*Duox, *Mj*Duox and *Dm*Duox) were predicted to have three EF-hand motifs, and the others were predicted to have two EF-hand motifs.

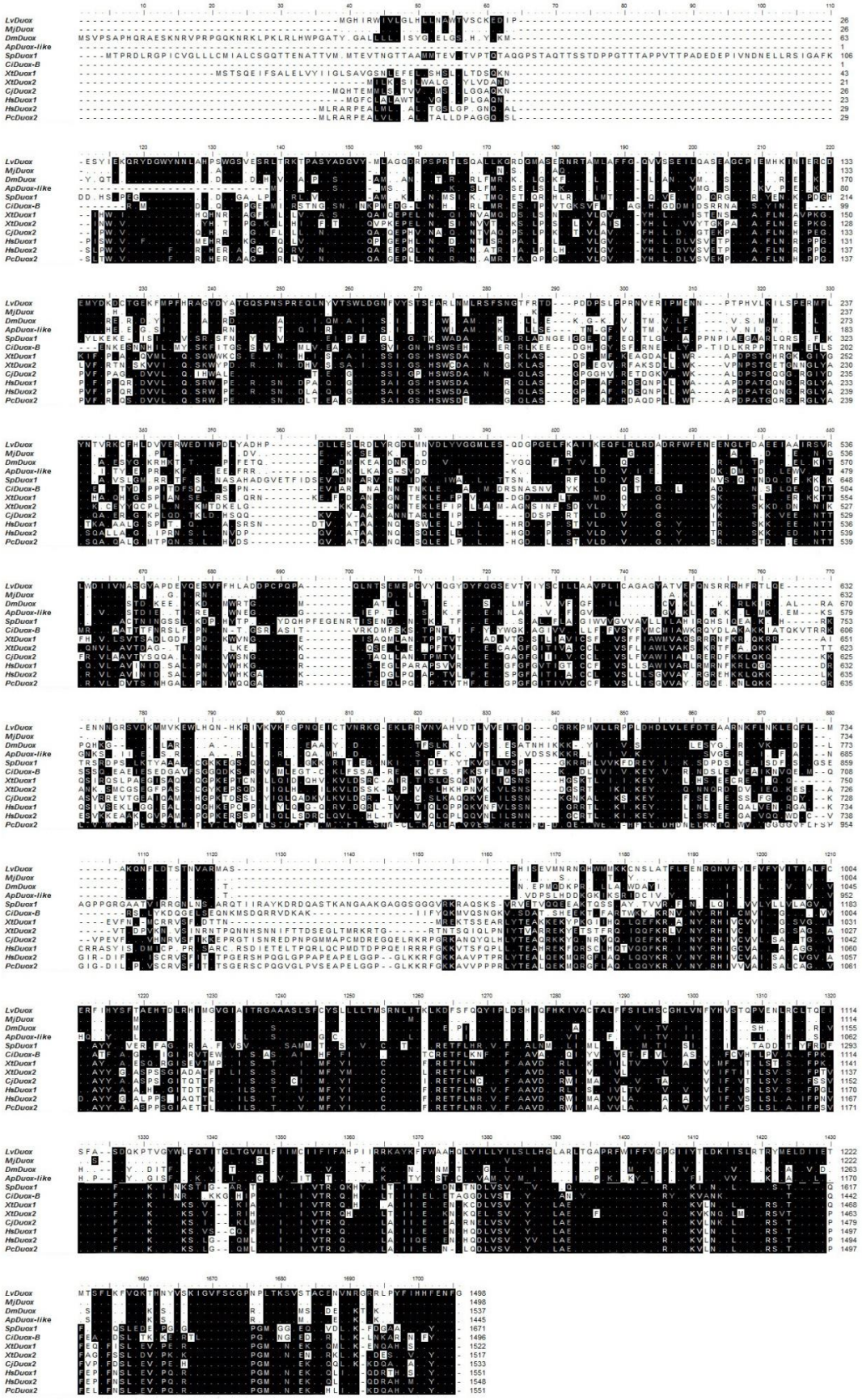

Fig. 3 Comparison of the amino acid sequence of dual oxidases from the Pacific white shrimp and the others organisms using the ClustalW program of BioEdit software. The full name, abbreviation and accession number are listed in the Table 3

Table 2 Amino acid identity of the Pacific white shrimp *Lv*Duox gene compared to the others known Duoxes sequences

Entire Duox	1	2	3	4	5	6	7	8	9	10	11	12
1.*Lv*Duox												
2.*Mj*Duox	96.1%											
3.*Dm*Duox	67.3%	67.0%										
4.*Ap*Duox-like	67.3%	66.9%	68.8%									
5.*Sp*Duox1	37.2%	37.3%	36.6%	35.6%								
6.*Ci*Duox-B	35.0%	35.1%	33.4%	34.5%	37.2%							
7.*Xt*Duox1	37.0%	36.6%	36.9%	35.5%	38.3%	43.5%						
8.*Xt*Duox2	36.2%	35.8%	35.1%	35.7%	35.6%	40.6%	56.6%					
9.*Cj*Duox2	38.1%	37.9%	37.5%	36.3%	39.4%	43.6%	61.5%	57.9%				
10.*Hs*Duox1	39.2%	39.3%	37.3%	36.0%	39.0%	42.2%	60.5%	56.2%	64.7%			
11.*Hs*Duox2	37.9%	37.8%	36.1%	35.0%	39.2%	41.8%	59.7%	56.4%	65.8%	77.2%		
12.*Pc*Duox2	37.6%	37.8%	36.4%	34.3%	38.7%	42.5%	60.0%	56.0%	65.9%	74.8%	87.4%	

Sequence homology and phylogenetic analysis

Sequence alignment was performed to determine the sequence identity of *Lv*Duox compared with the other Duox proteins (Fig. 3). *Lv*Duox shares 96.1% sequence similarity with *Mj*Duox, 67.3% with the insect Duox (*Dm*Duox and *Ap*Duox-like), 35% with the chordate Duox (*Ci*Duox-B) and 36.2–39.2% with the other Duoxs (Table 2).

To elucidate the evolutionary relationships between *Lv*Duox and other Duoxs, a neighbor-joining phylogenic tree was constructed by using sequence alignments in MEGA software (Fig. 4). In this phylogenic tree, *Lv*Duox formed a cluster with arthropod Duoxs, including *Mj*Duox, *Dm*Duox, *Tc*Duox and *Cf*Duox. The *Xt*Duox2, *Bt*Duox2 and the other Duoxs formed another cluster.

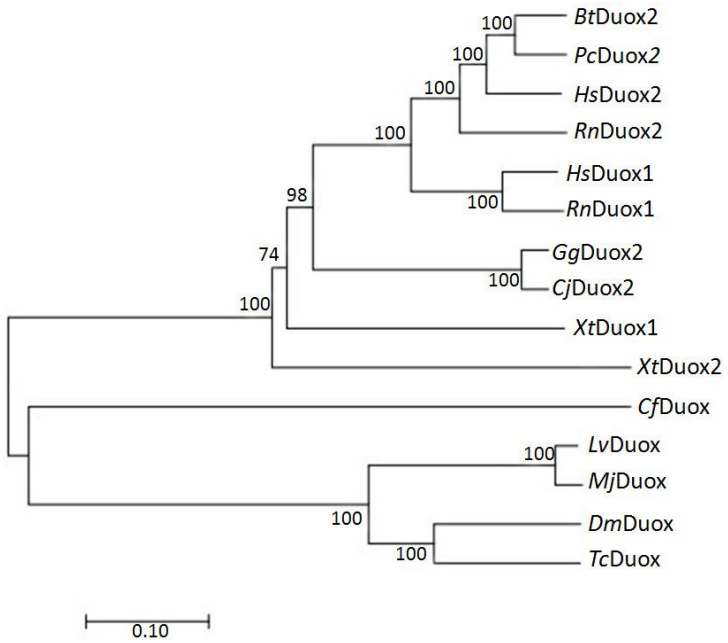

Fig. 4 The Neighbor-Joining (NJ) phylogenic tree constructed using MEGA 7.0 software package based on the amino acid sequences of Duoxs from different organisms. The numbers at the forks indicated the bootstrap value. The scale bar represents the proportion of amino acid differences between sequences based on nucleotide substitutions per site. The species and protein sequences ID are listed in Table 3

Table 3 Amino acid sequence numbers, symbols, GenBank accession numbers and nomenclatures used in the paper

Symbol	Accession number	Nomenclature
1.*Lv*Duox	MG734366	*Litopenaeus vannamei*
2.*Mj*Duox	AB744213	*Marsupenaeus japonicus*
3.*Dm*Duox	NP_608715	*Drosophila melanogaster*
4.*Sp*Duox1	NP_001118237	*Strongylocentrotus purpuratus*
5.*Dr*Duox1	BAF33370	*Danio rerio*
6.*Cj*Duox2	XP_015727798	*Coturnix japonica*
7.*Hs*Duox1	AAI14939	*Homo sapiens*
8.*Hs*Duox2	EAW77288	*Homo sapiens*
9.*Bt*Duox2	DAA25263	*Bos taurus*
10.*Rn*Duox1	AAN33120	*Rattus norvegicus*
11.*Rn*Duox2	NP_077055	*Rattus norvegicus*
12.*Gg*Duox2	XP_425053	*Gallus gallus*
13.*Xt*Duox1	XP_002937936	*Xenopus (Silurana) tropicalis*
14.*Xt*Duox2	XP_002937935	*Xenopus (Silurana) tropicalis*
15.*Cf*Duox	EFN70161	*Camponotus floridanus*
16.*Tc*Duox	XP_970848	*Tribolium castaneum*
17.*Ap*Duox-like	XP_001951113	*Acyrthosiphon pisum*
18.*Ci*Duox-B	FAA00329	*Ciona intestinalis*
19.*Bm*Duox	JQ768349	*Bombyx mori*
20.*Pc*Duox2	XP_007121449	*Physeter catodon*

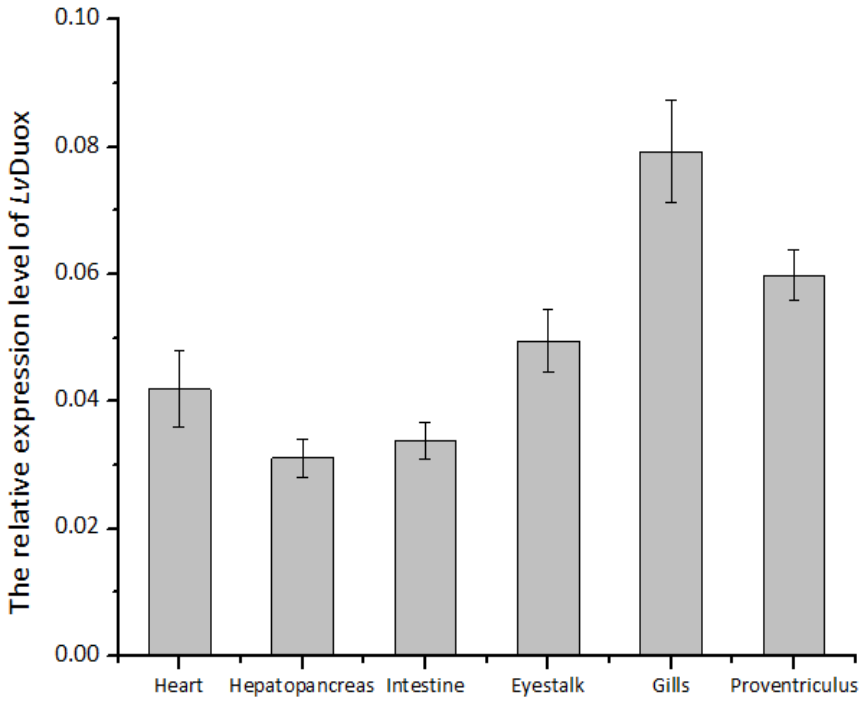

Fig. 5 Pacific white shrimp *Lv*Duox expression in various tissues of healthy shrimps (n=3). Tissue distribution of cDNA of *Lv*Duox was detected using quantitative real-time PCR. *β*-actin gene was used as the reference gene, and vertical bars represented mean ± SD

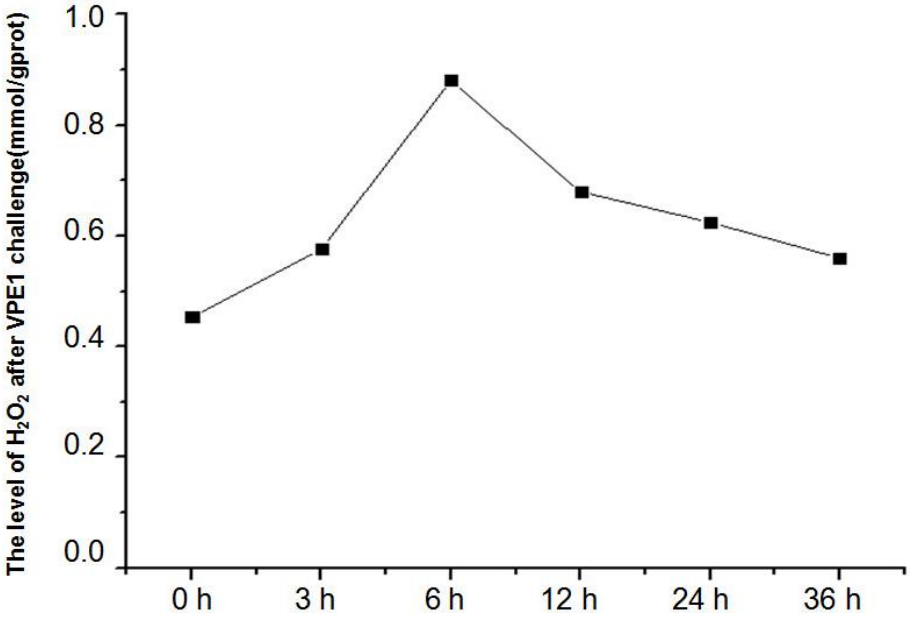

Fig. 6 The levels of the H_2O_2 in the midgut of the Pacific white shrimp following affected the *V. parahaemolyticus* E1 (VPE1). The level of the H_2O_2 was detected at different hours (0-36 h) using a hydrogen peroxide assay kit according to the manufacturer's instructions

Analysis of Lv*Duox expression in various tissues*

The qRT-PCR was used to detect the tissue distribution of *Lv*Duox gene expression, by using the *β*-actin gene as a reference. The expression levels of the *Lv*Duox gene were observed in different tissues, such as the heart, hepatopancreas, intestine, eyestalk, gills and proventriculus. The results showed that the expression of *Lv*Duox was higher in the gills than in the other tissues (Fig. 5).

H_2O_2 levels in the midgut after VPE1 challenge

Before VPE1 challenge, H_2O_2 was present at a low level in the shrimp midgut (0.45 mmol/g prot). After VPE1 stimulation, it increased at 3 h and peaked at 6 h (0.88 mmol/g prot), then declined gradually afterward (Fig. 6); the results were consistent with the expression of the *Lv*Duox gene (Fig. 7).

Analysis of expression of Lv*Duox and antioxidant genes after VPE1 challenge*

During VPE1 challenge, the midguts of Pacific white shrimps were obtained at 0 h, 3 h, 6 h, 12 h, 24 h and 36 h. The expression levels of the *Lv*Duox gene, superoxide dismutase (SOD) gene and catalase (CAT) gene in the shrimp midgut were determined using quantitative real-time PCR (Fig. 7). The relative gene expression level of *Lv*Duox in the midgut increased significantly at 3 h (5.03+0.41) and 6 h (5.33+0.4) (P<0.05), then decreased gradually at 12–36 h. The expression of the SOD gene decreased 3–6 h after the VPE1 challenge, then began to increase at 12 h (2.16+0.21), peaked at 24 h (3.53+0.22) (P<0.05) and then decreased significantly at 36 h. The expression level of the CAT gene at 6–12 h was lower than that at 0–3 h, but significantly increased at 24 h (2.32+0.28) (P<0.05), then decreased to normal levels.

Discussion

Duox has been studied extensively in many model species, but there have been few reports in commercial aquatic animals. The role of Duox in the innate immunity of the Pacific white shrimp remains unknown. In this study, the full-length sequence of the Duox gene of the Pacific white shrimp was cloned and named *Lv*Duox, and was deposited in GenBank under accession number MG734366 (not released). The ORF was 4,497 bp and encoded a 1,498 amino acid protein with a theoretical mass of 173 kDa, results similar to those for *Marsupenaeus japonicus* (~173 kDa), *Bombyx mori* (~172 kDa), *Danio rerio* (~172 kDa) and *Drosophila melanogaster* (~178 kDa). The amino acid sequence of *Lv*Duox has a higher identity to Duox from arthropods than from other species. Both structural domain comparison and sequence alignment indicated that *Lv*Duox was more similar to crustacean *Mj*Duox than to other Duoxs (Inada *et al.*, 2013). Thus, the Duox gene appears to be highly conserved in different kinds of shrimp.

The analysis of structural domains of *Lv*Duox revealed that a peroxidase domain, the transmembrane segment, the calcium binding region, a ferric reduction region, a FAD binding domain and a NADPH binding domain were conserved, and the signal peptide also was present in many Duoxs expected for *Bm*Duox and *Dr*Duox1. There was a coiled coil in *Lv*Duox and *Mj*Duox. The coiled coil is a structural motif in proteins, in which 2-7 alpha-helices coil together like the strands of a rope,

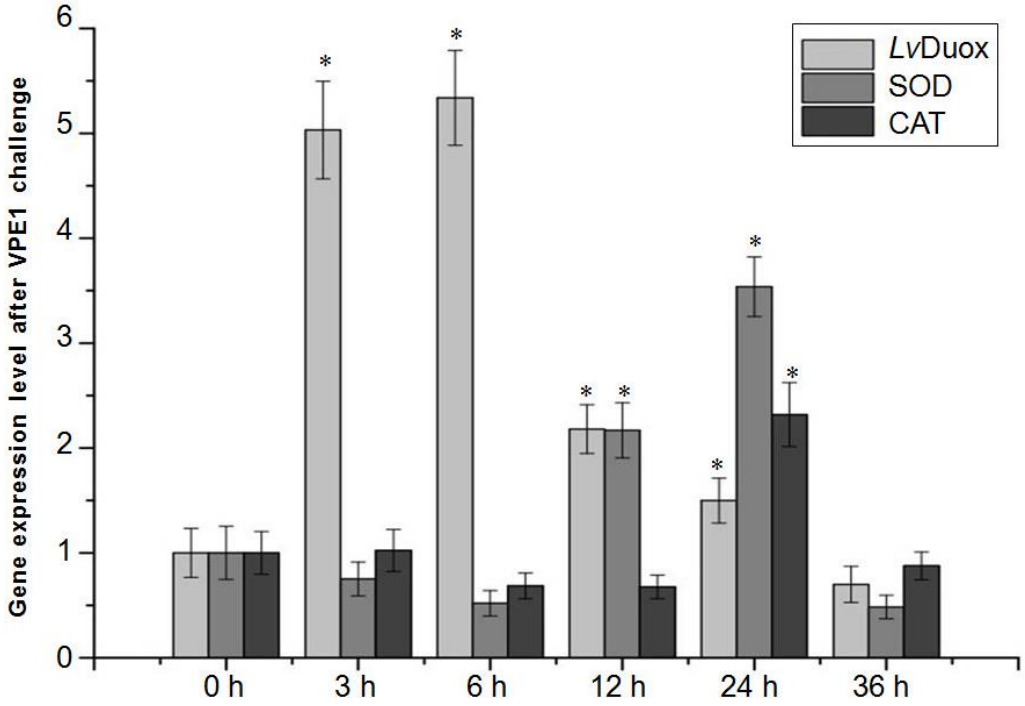

Fig. 7 The expression of the genes (*Lv*Duox,SOD and CAT) in the midgut of the Pacific white shrimp following affected the *V. parahaemolyticus* E1 (VPE1). The expression levels of the genes were detected at different hours (0-36 h) using quantitative real-time PCR, and *β*-actin gene was used as the reference gene, and differences were considered significant at *P<0.05

and dimers are common. The coiled coil plays a major role in cell recognition and signal transduction. Therefore, the coiled coil may be a special domain distinguishing the Duoxs of shrimps from those of other organisms.

The NADPH oxidase domain can produce H_2O_2, whereas the peroxidase domain can convert H_2O_2 into HOCl. H_2O_2 and HOCl aid in resistance to the intrusion of pathogens and provide an important immune defense mechanism in organisms that is necessary for the adaptive immune response. The calcium binding region formed by three EF-hand motifs was predicted in three arthropod Duoxs (*Lv*Duox, *Mj*Duox and *Bm*Duox), whereas the others contained two EF-hand motifs (Fig. 2). Intracellular concentrations of Ca^{2+} modulate *Bm*Duox enzymatic activity via the EF-hand motifs (Hu *et al.*, 2013). Thus, we believe that the EF-hand motifs of *Lv*Duox may be involved in the response to Ca^{2+} in a manner similar to the mechanism in the fruit fly.

The mRNA transcripts of *Lv*Duox gene were observed in all the detected tissues. *Lv*Duox had high expression in the gills, a respiratory organ that, like the intestine, directly contacts water and bacteria. In the midgut of shrimp infected by VPE1, the expression of *Lv*Duox increased significantly at 3 h after infection (P<0.05), peaked at 6 h (P<0.05), then began to decline and returned to its original level at 36 h. The trends in H_2O_2 levels in the midgut were consistent with the expression level of the *Lv*Duox gene. VPE1 stimulated the expression of *Lv*Duox, and H_2O_2 from *Lv*Duox participated in resisting the invasion of VPE1. As the SOD and CAT gene expression increased significantly between 12 and 24 h (P<0.05), the level of H_2O_2 declined gradually. We concluded that the high concentration of ROS in the midgut induced the response of the antioxidant system to protect the organism from oxidative damage.

Initial research has revealed that the production of O_2^- in the hemocytes of Pacific white shrimps is dependent on the concentration of bacteria (*Escherichia coli*) (Munoz *et al.*, 2000). In addition, the expression of kuruma shrimp *Mj*Duox increases after white spot syndrome virus injection (Inada *et al.*, 2013). Thus, our results suggested that foreign pathogens stimulate the expression of *Lv*Duox to participate in innate immunity, and the antioxidant genes regulate H_2O_2 levels, thus protecting the shrimp against oxidative damage induced by ROS.

In conclusion, we cloned the full-length cDNA encoding *Lv*Duox. On the basis of sequence alignment and phylogenetic analysis, the *Lv*Duox was found to be highly conserved and to be more similar to arthropod Duoxs than to vertebrate and echinoderm Duoxs. The *Lv*Duox gene was expressed in all the main organs of the white shrimp and responds to invading pathogenic bacteria in the midgut. Two antioxidant genes were involved in the regulation of H_2O_2 levels generated by *Lv*Duox. Therefore, *Lv*Duox may be a new target for intestinal disease research. More studies are needed to clarify the regulatory mechanism of *Lv*Duox in the innate immunity system and to determine how to accurately control the expression of Duox in shrimp to protect cells from ROS damage.

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Competing financial interests

The authors declare no competing financial interests.

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