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Short communication

Transcriptome analysis in the skin of *Carassius auratus* challenged with *Aeromonas hydrophila*

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ABSTRACT

Skin plays an important role in the innate immune responses of fish, particularly towards bacterial infection. To understand the molecular mechanism of mucosal immunity of fish during bacterial challenge, a *de novo* transcriptome assembly of crucian carp *Carassius auratus* skin upon *Aeromonas hydrophila* infection was performed, the latter with Illumina Hiseq 2000 platform. A total of 118111 unigenes were generated and of these, 9693 and 8580 genes were differentially expressed at 6 and 12 h post-infection, respectively. The validity of the transcriptome results of eleven representative genes was verified by quantitative real-time PCR (qRT-PCR) analysis. A comparison with the transcriptome profiling of zebrafish skin to *A. hydrophila* with regards to the mucosal immune responses revealed similarities in the complement system, chemokines, heat shock proteins and the acute-phase response. GO and KEGG enrichment pathway analyses displayed the significant immune responses included TLR, MAPK, JAK-STAT, phagosome and three infection-related pathways (ie., *Salmonella*, *Vibrio cholerae* and pathogenic *Escherichia coli*) in skin. To our knowledge, this study is the first to describe the transcriptome analysis of *C. auratus* skin during *A. hydrophila* infection. The outcome of this study contributed to the understanding of the mucosal defense mechanisms in cyprinid species.

1. Introduction

The crucian carp *Carassius auratus* (*Cyprinidae*) is a major freshwater species cultured for human consumption in China, with an annual production of over 3.0 million tons in 2016 [1]. In recent years, fish skin ulcerations and haemorrhagic septicaemia caused by *Aeromonas hydrophila* has led to great economic losses during mass production in China [2–4]. To date, exploration of skin immunity at the molecular level of the crucian carp is scarce. In this context, it is crucial to understand how this fish and other cyprinid species resist and/or respond to *Aeromonas hydrophila* infection and the mechanistic actions involved during activation of the innate and adaptive immune systems in order to formulate strategies to battle pathogenic bacteria challenge during infection.

Skin plays an important role in the innate immune responses of fish and acts as the first line of defense during infection, including pathogenic bacteria [5–7]. Skin is a component of the mucosal immune system and a few studies on the skin transcription profile were characterized in fish [8–10]. Skin transcriptome analyses of Sea trout (*Salmo truttam*) and Mud Loach (*Misgurnus anguillicaudatus*) provided evidence that the genes involved in immunity and epidermal mucus secretion show a complexity of skin immune mechanism [9,10]. The transcriptional profiles of the Atlantic cod (*Gadus morhua*) skin displayed high expression of antibacterial activity and antiviral response genes [8]. Recently, studies into the skin of zebrafish (*Danio rerio*) [11] and grouper (*Epinephelus coioides*) [12] revealed that the mucosal immune response is classified into several subgroups including complements, chemokines, acute-phase response, as well as antigen processing and presentation upon infection by various pathogens. However, to date, very little is known about the molecular immune response of skin towards infectious pathogens in fish [11,12]. In this study, the transcription profile of the crucian carp skin using an *A. hydrophila* early infection-by-immersion model which mimics the natural infection route was constructed based on the sequence analysis generated by the Illumina Hiseq 2000 system, with the aim of clarifying the mechanism of skin immune responses against bacterial pathogens in fish.

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Abbreviations

APR	Acute phase response
C7	Complement 7
COG	Cluster of orthologous groups
CRP	C reactive protein
DEG	Differentially expressed gene
GO	Gene ontology
HSP	Heat shock protein
IRF	Interferon regulatory factor
IFN	Interferon
IkBα	Inhibitor of nuclear factor-kappa B alph
IL-22	Interleukin-22
JAK-STAT	Janus kinase-signal transducer and activator of transcription
KEGG	Kyoto encyclopedia of genes and genomes
MAPK	Mitogen-activated protein kinase
MHC	Major histocompatibility complex
Mx	Interferon inducible GTP binding protein gene
NF-κB	Nuclear factor kappa B
NOD	Nucleotide-binding oligomerization domain
PAMP	Pathogen-associated molecular pattern
RSAD2	Radical S-adenosyl methionine domain-containing protein 2
SF3B3	Splicing factor 3B subunit 3
TRAF6	Tumor necrosis factor receptor associated factor 6
TLR	Toll-like receptors
TCR	T-cell receptor
TNF	Tumor necrosis factor

2. Materials and methods

2.1. Aeromonas hydrophila infection in crucian carp

All procedures involving the handling and treatment of fish used during this study were approved by the Henan Normal University Institutional Animal Care and Use Committee (HNU-IACUC) prior to initiation. Crucian carp were infected with *A. hydrophila* by immersion as previously described [13]. Crucian carp (average weight 50 ± 5 g, body length 13 ± 2 cm) were acclimatized at 25 ± 2 °C in 45 L tanks with aerated freshwater for 15 days. Fish were fed with commercial dried pelleted feed (Tongwei, China) twice daily. For the challenge, fish were immersed in 1.0×10^8 cfu mL^{-1} *A. hydrophila* for 3 h and subsequently transferred to freshwater. Control fish were incubated in freshwater. At 6 and 12 h post-infection (pi), 60 control and infected fish were randomly selected, and skin sections (10 mm^2 area per section) were aseptically excised from the dorsal regions along both sides of the fish. Triplicate pooled skin samples were immediately frozen in liquid nitrogen and then stored at −80 °C for RNA extraction.

The skin, intestine, liver and kidney tissues of the infected crucian carp with typical clinical signs at 24 hpi were fixed in Bouin's fixative at room temperature. Serial sections (5 μm thick) of paraffin-embedded tissues were cut using a microtome (Thermo) and stained with haematoxylin and eosin (H&E) for further examination under a light microscope (Leica). In addition, the tissues of the fish treated with physiological saline were used as controls.

2.2. RNA extraction, RNA-Seq library construction and sequencing

Total RNA was extracted from the skin samples using Trizol reagent (Invitrogen) and was further purified with RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. The purity, quantity and integrity of total RNA were determined using NanoDrop ND-2000 (NanoDrop), and an Agilent Bioanalyzer 2100 (Agilent). Approximately 5 μg total RNA was used for RNA-Seq library construction using the TruSeq RNA sample preparation kit (Illumina). Crucian carp skin Hiseq sequencing library of control group (tSC), the infected group at 6 h (ST1) and 12 h (ST2) were constructed by using an Illumina Hiseq 2000 platform with instruments in pairs, making the usual 90 bp readings.

2.3. De novo assembly and annotation

Transcriptome *de novo* assembly is carried out with short reads assembling program Trinity package. The raw data generated from Illumina sequencing were first quality-filtered to eliminate adaptor sequences, low-quality bases ($Q < 20$) and unpaired reads [14]. Based on the short reads-assembling Trinity package with a k-mer length of 25, the final clean reads were *de novo* assembled into contigs. The assembled contigs of all the samples were cleaned by removing redundancies and were further assembled as primary unigenes, and the final clean reads were aligned to the primary unigenes as described [12]. Primary unigenes with at least 10 mapped reads were considered reliable unigenes. Blast2GO program was used to annotate the entire unigenes based on the NCBI non-redundant (Nr) database [15]. The KEGG pathway and COG annotations were performed using blastx search against the Nr, Swiss-Prot, KEGG and COG databases. GO functional classification for unigenes was performed according to the previous report [16].

2.4. DEGs identification and validation

All unigenes were used as a reference transcriptome for downstream analysis. The expression level of the gene was measured using fragments per kilobase of transcript per million fragments mapped (FPKM). The values were calculated for further analysis of identify DEGs among the three groups using edgeR software [17]. DEGs were further functionally analyzed by GO term enrichment and KEGG pathway enrichment. Only gene expressions with more than 2.0-fold or less than −2.0-fold change were considered significant regulation in skin immune responses.

De novo transcriptome data were validated by quantitative real-time PCR (qRT-PCR), and association analysis of the transcriptome and isobaric tags for relative and absolute quantification (iTRAQ) - based proteome was performed according to the previous report [18,19]. Primers were designed using Primer5 software and obtained from Sangon Biotech (Table S1). qRT-PCR was performed according to the previous report [20], and the expression of the candidate genes was normalized using β-actin as a housekeeping gene. Each gene was run in triplicate. After completion of the qRT-PCR amplification, the relative fold changes after infection were calculated based on the $2^{-\Delta\Delta Ct}$ method [21].

3. Results

3.1. Clinical signs and histopathology in the skin of crucian carp

Clinically diseased fish infected with *A. hydrophila* exhibited skin hemorrhage, abdominal swelling and congestion of visceral organs, and the histopathology revealed an eroded epidermis, fused and hypertrophied mucous cells, and dense inflammatory infiltrates as well as hemorrhagic lesions in the dermis (Fig. 1). Different degrees of pathological changes in fish intestine, liver and kidney were observed after *A. hydrophila* challenge.

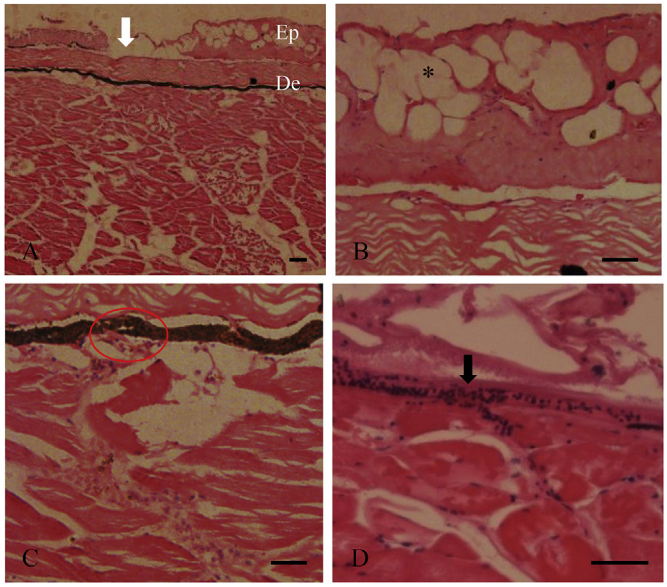

Fig. 1. Histopathology of skin from crucian carp infected with *A. hydrophila*. (A) infected skin displayed severe epithelial ulceration (white arrows, scale bar 100 μm), (B) hypertrophied mucous cells (asterisk, scale bar 50 μm), (C) melanocytes and melanin deposits and hemorrhagic lesions (round, scale bar 50 μm), (D) inflammatory cell infiltrates (black arrows, scale bar 20 μm). Ep is epidermis and De is dermis, the sampling time of fish tissues was at 24 hpi.

3.2. Skin transcriptome analysis of crucian carp

The de *novo* transcriptome of *C. auratus* skin post *A. hydrophila* infection revealed a total of 118111 unigenes, with the mean size and N50 occurred at 958 bp and 1846 bp, respectively. For function annotation analysis, 60824, 91271, 51143, 43269, 17827 and 37103 unigenes were annotated to the NR, NT, Swiss-Prot, KEGG, COG and GO database, respectively, with the total annotation unigenes of 94776 (Table 1). Samples ST1 (6 h) and ST2 (12 h) respectively generated 125242 and 119022 unigenes. A total of 9693 and 8580 DEGs in ST1 and ST2 skin samples. Of these, 4581 (6 h), 3220 (12 h) genes were up-regulated while 5112 (6 h), 5360 (12 h) genes were reduced significantly in ST1 and ST2 skin samples upon infection, respectively (Fig. S1). As revealed by AmiGO 2 database (Immune response, GO: 0006955), a total of 129 and 108 immune response-related unigenes were expressed at 6 and 12 h post-infection, respectively. Eight complement components (c1, c1ql2, c7, ce, cg, crmp, cr1, cfh from 1.577 to 5.772-folds) were up-regulated, and the expression of both complement receptor cr1 (from 3.823 to 4.169-fold) and complement regulatory protein (> 5.772-fold) were enhanced significantly upon early stage of *A. hydrophila* infection. Conversely, some genes linked to antigen processing and presentation (from −1.89 to −12.43-folds), possibly function in MHC antigen presentation were down-regulated. In addition, several key molecules such as interferon regulatory factor-1 (irf-1, > 3.05-folds), Hsp70 and Hsp90 (> -13.396-folds), alpha-2-macroglobulin (from −3.217–2.494 -folds) and two caspases (> -4.594-folds) related to the proteolysis and trafficking of exogenous and endogenous proteins during antigen processing and presentation were identified. Some IFN-inducible genes (up-regulated from 3.351 to 15.75 -fold) and ILs (up-regulated from 1.808 to 6.366-fold) were found to be involved in skin immune responses. It was further enriched in two DEGs groups (ST1, ST2) suggesting an important role of the MAPK signaling pathway (eg, map2k2 from −1.547 to −3.650-fold, mapk5 from 2.22 to 2.722-fold, map3k13 from −4.31 to −2.387-fold, mapkg1 from −2.188–1.789-fold) in skin immune responses of crucian carp. Besides, several unknown function unigenes such as LOC100332375, LOC100698102, LOC393377, LOC767810, LOC550603, LOC100038770, Zgc:171497 and zgc:77326 were significantly differentially expressed in the skin of the crucian carp upon infection (Table S2).

3.3. Association analysis for transcriptome and proteome and validation of DEGs in crucian carp skin

To investigate whether co-association of same genes/proteins is differentially expressed between transcriptome and proteome, the association of differential expression profiles were further analyzed in the skin of the crucian carp. The transcriptome (RNA-Seq) and iTRAQ-based quantitative proteomic analyses showed that the mRNA and protein expression only for 18 and 10 unigenes at 6 h and 12 h post-infection were correlated. To further validate the transcriptome analysis, eleven immune-related genes (ie., MHC-I, MHC-II, RSAD2, Mx1, TNF-β, TRAF6, IkBα, C7, CRP-2, IL-22 and SF3B3) were selected for qRT-PCR analysis (Fig. 2). Relative fold changes of qRT-PCR were compared using the RNA-Seq expression profiles, and linear regression analysis showed that the fold changes in the gene expression ratios of the RNA-Seq and the qRT-PCR data were correlated (Pearson's correlation coefficient = 0.903, $p < 0.01$).

3.4. GO terms and KEGG pathway enrichment analyses

GO category enrichment analysis of DEGs showed that cellular process, metabolic process, biological regulation and response to stimulus were the four most abundant GO term in the crucian carp skin. The antigen processing and presentation, cell adhesion, complement activation, blood coagulation, chemokines and apoptosis were mainly involved in skin immune responses to *A. hydrophila* infection (Fig. 3). For functional prediction and COG database, in total 17827 unigenes were classified into 24 functional categories (Table 2). KEGG enrichment analysis showed that the 28 significant immune response pathways included phagosome, MAPK, TLR, NF-kB, NOD-like receptor, natural killer cell mediated cytotoxicity, TCR and JAK-STAT in skin immune responses (Table 3). The DEGs involved in antigen processing and presentation, complement and coagulation cascades, TLR, and MAPK signaling pathway were shown in Fig. S2. Moreover, three infection-related pathways of *Salmonella*, *Vibrio cholerae* and *Escherichia coli* were identified in the crucian carp skin immunity association based on KEGG enrichment analysis (Fig. 4).

4. Discussion

In recent years, fish skin ulcerations and haemorrhagic septicaemia caused by *A. hydrophila* has led to great economic losses in aquaculture [2–4]. Skin is one of the gateways between fish body and external water environment, but its molecular mechanism of mucosal immune response remains unclear in crucian carp [11,12]. In this study, the skin

Table 1
Statistical summary of *C. auratus* skin transcriptome data.

Sequencing	Number of reads	118,111
Assembly	number of high quality reads	16,176
	percentage of assembled reads	13.70%
	number of contigs (nt)	241,723
	mean length of the contigs (nt)	958
	N50 (nt)	1846
	number of unigenes	94,776
	GC content	44.62%
Annotation (database)	NCBI nr	60,824 (25.16%)
	NT	91,271 (37.76%)
	Swiss-Prot	51,143 (21.16%)
	KEGG	43,269 (17.90%)
	COG	17,827 (7.37%)
	GO	37,103 (15.35%)

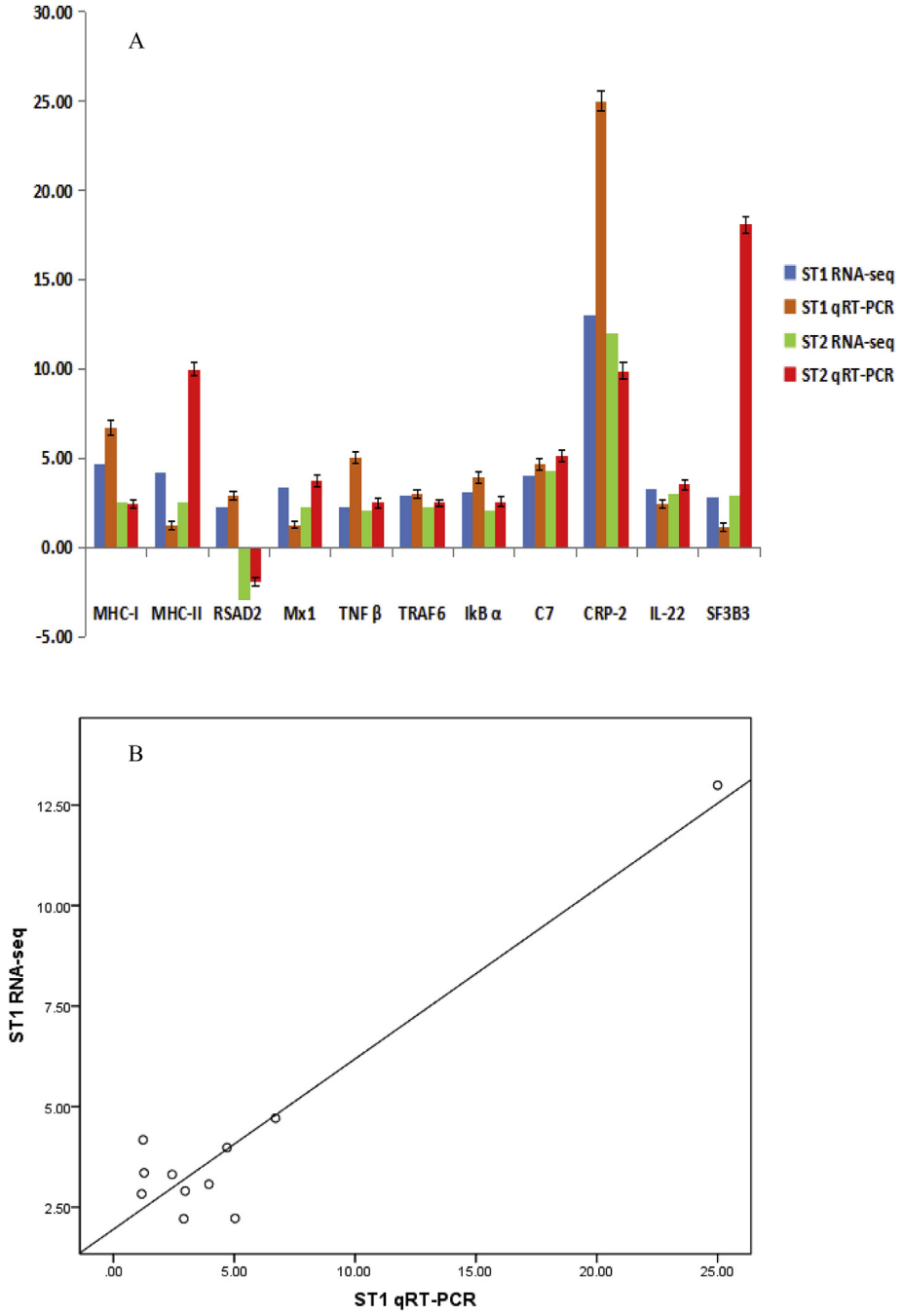

Fig. 2. Validation of RNAseq-based gene expression profiles by qRT-PCR. (A) Eleven immune-related genes were selected, and fold changes were determined by calculating the ratio of the mean expression values from the control and infected skin samples, (B) Correlation of the relative fold changes in gene expression between the RNA-seq and qRT-PCR analyses at 6 h. Each black dot indicates the relative gene expression fold change according to RNA-seq and qRT-PCR results. Three technical replicates were performed for each biological replicate.

transcription profile of the crucian carp against pathogen *A. hydrophila* was identified in order to explore the immune responses of carps against bacterial pathogens.

Several studies have indicated the classical and alternative pathways of the complement system during pathogen encounter [22,23]. A study on the skin transcriptome analysis of *C. irritans* infected grouper [12], *A. hydrophila* and *Citrobacter freundii* infected zebrafish [11,20] revealed the robust synthesis of complement components genes involved in fish immune response. In this study, the differentially expression of complement-related genes were found in crucian carp skin, indicating that these genes may probably activate the complement pathways and immune response of the skin.

Antigen processing and presentation is responsible for presenting antigen to lymphocytes, and TCR participate in antigen recognition peptide-MHC to activate and regulate humoral and cellular immune response [24–26]. In catfish [25], Atlantic salmon [26] and zebrafish [11,20] MHC class I and MHC class II genes were differentially expressed upon infection and in this context, MHC related-genes in the skin of carp with *Trypanoplasma borreli* [27], rainbow trout with *Gyrodactylus derjavini* [28] and in gills of Atlantic salmon were down regulated upon pathogenic challenge [29]. Interestingly, several antigen processing and presentation molecules such as adaptor protein complex AP-1 subunit beta-1, BOLA class I histocompatibility antigen and hypothetical protein LOC100698102 were shown to be up-

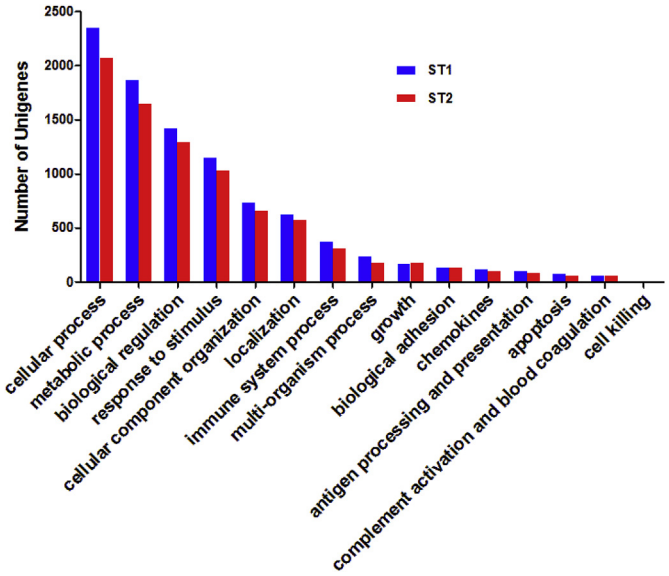

Fig. 3. GO analysis of the DEGs in the skin of crucian carp based on biological process.

regulated, and enhanced accumulation of these genes in crucian carp skin may eliminate invading pathogens as part of the acute immune response.

TLRs represent the first line of immune activation and they play crucial roles in the pathogen recognition, otherwise known as the PAMPs [30–32]. Activation of TLR3 signaling induces the expression and secretion of cytokines TNF-α, IL-1β and IL-8, and their activation contributes to the host immunity in fish [31,32]. In fish skin, TLRs genes were differentially expressed in channel catfish upon infection with *Ichthyophthirius multifiliis* [33], miiuy croaker (*Miichthys miiuy*) and yellow croaker (*Pseudosciaena crocea*) upon infection with *V. anguillarum* [34]. TLRs pathway was also involved in grouper skin immune response [12]. In this study, TLRs were regulated from −2.92–3.20-folds in the skin of the crucian carp, indicating that the immune response of this piscine species is quite similar to the species as just described. MAPK signaling pathways are involved in cell growth, apoptosis, inflammation and response to environmental stresses [35]. Five genes were involved in MAPK signaling pathway in zebrafish (*D. rerio*) infected with *C. freundii* [20] and a total of 19 genes were identified during *A. hydrophila* infection [11]. In our present results, the differential expression of MAPK-related genes (from −3.65–6.11-fold) suggested that MAPK signaling pathways be involved in the skin immune process.

Additionally, three bacterial infection pathways including *Salmonella, V. cholerae and* pathogenic *E. coli* infected pathways were found in this study, but few were reported in literatures [11,18]. *Salmonella* infection raises a globally important health problem in fish, and zebrafish have been regarded as a good fish model for *Salmonella* infection [36,37]. The proteomic analysis of gill proteins from the zebrafish infected with *A. hydrophila* found that pathogenic *E. coli* infection were the significant pathway in mucous immune reponses [19]. Here, the *A. hydrophila* infection pathway in the skin of crucian carp was described. Based on *Salmonella*, *V. cholerae* and *E. coli* infection pathways analyses, the hypothetic pathway of *A. hydrophila* infection will contribute to understanding the molecular mechanisms of the skin immunity in fish.

The expression of immune-related genes in zebrafish upon bacterial infection is quite similar to those observed in the crucian carp. APR related-genes including transferrin, ceruloplasmin, alpha-2-macroglobulin, vitellogenin and apolipoprotein were robustly upregulated in the skin [11,20], in addition to IFNs, a group of transcriptionally regulated cytokines which are involved in innate immune response [38,39]. IFN binds to the receptors on nucleated host cells, and activate JAK-STAT signal transduction, and thereby regulate downstream genes in the IFN pathway [39,40]. The bacterial infection resulted in significant up-regulation of IFN and MHC class I gene expression in zebrafish skin, supplying additional evidence that MHC class I is transcriptionally activated by IFN [11,20,41]. Besides, active forms of ILs could lead to the activation of type I IFN response and induce apoptosis of infected cells, and is involved in the immune regulation in fish [42,43]. Studies in the golden pompano (*Trachinotus ovatus*) [44] and common carp [45] revealed that IL-22 is potentially involved bacterial attenuation, observation similar to the ones observed in this study.

In summary, the transcriptome profiling of DEGs in the skin of

Table 2
COG classification of the unigenes from the skin of crucian carp.

COG category	COG functional class	Number of contigs
Information storage and processing	Translation, ribosomal structure and biogenesis [J]	3011 (7.34%)
	RNA processing and modification [A]	245 (0.59%)
	Transcription [K]	3495 (8.48%)
	Replication, recombination and repair [L]	4148 (10.07%)
	Chromatin structure and dynamics [B]	504 (1.22%)
Cellular processes and signaling	Cell cycle control, cell division, chromosome partitioning [D]	2619 (6.36%)
	Nuclear structure [Y]	19 (0.05%)
	Defense mechanism [V]	124 (0.30%)
	Signal transduction mechanisms [T]	2631 (6.39%)
	Cell wall/membrane/envelope biogenesis [M]	1894 (4.60%)
	Cell motility [N]	487 (1.18%)
	Cytoskeleton [Z]	1083 (2.63%)
	Extracellular structures [W]	30 (0.07%)
	Intracellular trafficking, secretion, and vesicular transport [U]	1618 (3.93%)
	Posttranslational modification, protein turnover, chaperones [O]	2441 (5.92%)
Metabolism	Energy production and conversion [C]	740 (1.80%)
	Carbohydrate transport and metabolism [G]	1948 (4.73%)
	Amino acid transport and metabolism [E]	836 (2.03%)
	Nucleotide transport and metabolism [F]	342 (0.83%)
	Coenzyme transport and metabolism [H]	438 (1.06%)
	Lipid transport and metabolism [I]	746 (1.81%)
	Inorganic ion transport and metabolism [P]	628 (1.52%)
	Secondary metabolites biosynthesis, transport and catabolism [Q]	1024 (2.49%)
Other	General function prediction only [R]	8046 (19.53%)
	Function unknown [S]	2103 (5.10%)

Table 3
Immune-related pathways of crucian carp skin involving in response to *A. hydrophila*.

KEGG_pathway	Description	Count	*P*-value
		(6 h/12 h)	(6 h/12 h)
ko04145	Phagosome	175/133	3.86e-14/2.11e-06
ko04514	Cell adhesion molecules	142/120	1.76e-13/1.24e-09
ko04110	Cell cycle	136/138	3.16e-13/2.45e-17
ko04064	NF-kappa B signaling pathway	136/83	9.36e-14/9.41e-03
ko04010	MAPK signaling pathway	135/107	0.46/0.92
ko04080	Neuroactive ligand-receptor interaction	131/158	3.64e-09/7.14e-22
ko04621	NOD-like receptor signaling pathway	120/114	1.03e-08/1.77e-09
ko05132	Salmonella infection	99/73	0.07/0.71
ko04620	Toll-like receptor signaling pathway	95/57	1.43e-08/9.42e-02
ko04060	Cytokine-cytokine receptor interaction	94/92	8.38e-02/1.73e-02
ko04650	Natural killer cell mediated cytotoxicity	93/76	1.79e-04/9.48e-03
ko04512	ECM-receptor interaction	89/93	5.69e-03/4.68e-05
ko04670	Leukocyte transendothelial migration	88/58	0.53/1.00
ko05320	Autoimmune thyroid disease	79/61	4.88e-19/1.27e-11
ko04660	T cell receptor signaling pathway	75/64	0.53/0.71
ko04622	RIG-I-like receptor signaling pathway	72/53	2.16e-06/0.01
ko04020	Calcium signaling pathway	71/69	0.66/0.41
ko04630	Jak-STAT signaling pathway	67/40	0.39/1.00
ko04115	p53 signaling pathway	65/65	4.21e-05/1.75e-06
ko05130	Pathogenic *Escherichia coli* infection	62/59	0.78/0.63
ko05110	*Vibrio cholerae* infection	61/61	0.25/7.02e-02
ko04662	B cell receptor signaling pathway	61/48	0.15/0.53
ko04370	VEGF signaling pathway	45/33	0.68/0.69
ko04310	Wnt sinaling pathway	40/57	1.00/0.97
ko04350	TGF-BETA sinaling pathway	33/26	1.00/1.00
ko04150	mTOR sinaling pathway	28/27	0.97/0.94
ko04672	Intestinal immune network for IgA production	25/24	0.04/0.02
ko04330	Notch sinaling pathway	21/15	1.00/1.00

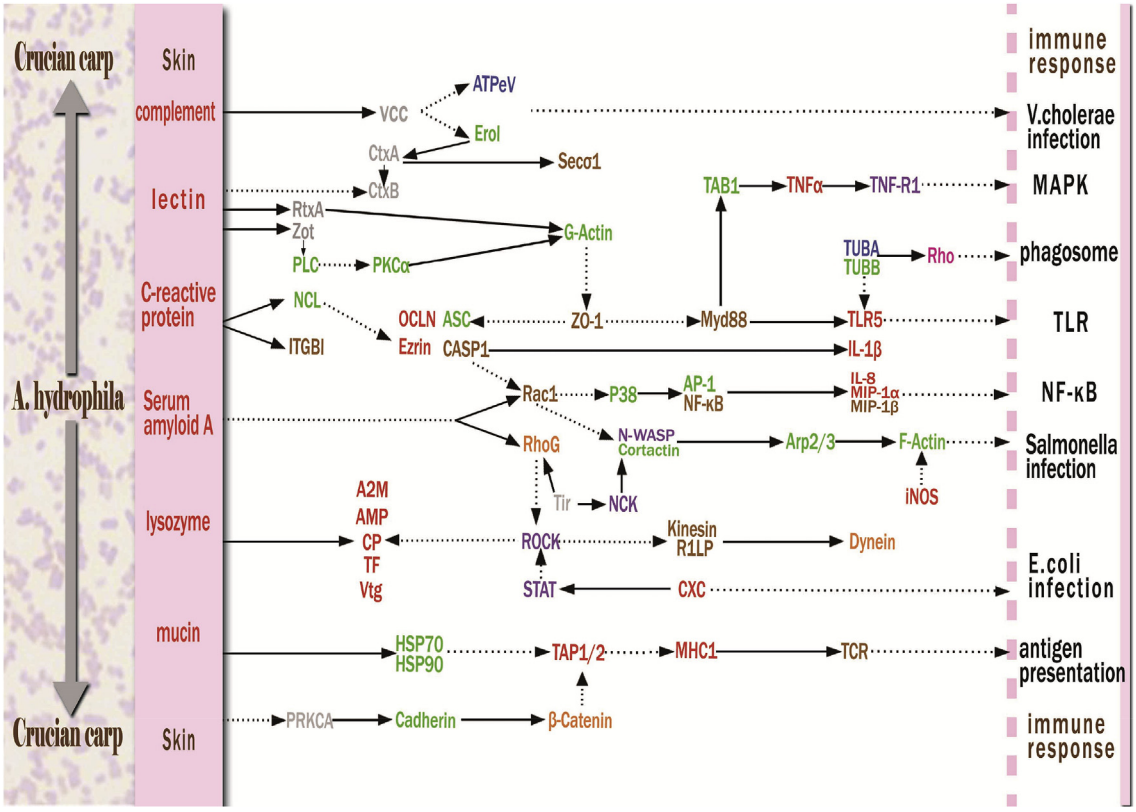

Fig. 4. Schematic diagram of signaling pathways in crucian carp skin induced by *A. hydrophila* infection. The DEGs for different pathways are shown in red (co-upregulated at both 6 h and 12 hpi), green (co-downregulated at both 6 h and 12 hpi), purple (upregulated at 6 h while downregulated at 12 hpi), blue (down-regulated at 6 h while upregulated at 12 hpi), brown and orange (upregulated or downregulated at 6 hpi). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

crucian carp revealed that antigen processing and phagosome, chemokines, apoptosis, MAPK, TLR, and three bacterial infection pathways are involved in the regulation of mucosal immune response. When compared with the transcription profiles of zebrafish skin to *A. hydrophila*, obvious similarities (eg. complements, chemokines, heat shock proteins, acute-phase response) were observed in mucosal immune responses [11]. The transcriptome analysis of *C. auratus* skin during *A. hydrophila* infection were described herein, and the outcomes are useful to further understand the mechanisms involved in the innate immune response of fish.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.09.039.

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