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Transcriptomic characterization of adult zebrafish infected with *Streptococcus agalactiae*

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ABSTRACT

Streptococcus agalactiae is a major aquaculture pathogen infecting various saltwater and freshwater fish. To better understand the mechanism of the immune responses to *S. agalactiae* in wildtype zebrafish, the transcriptomic profiles of two organs containing mucosal-associated lymphoid tissues from *S. agalactiae*-infected and non-infected groups were obtained using RNA-seq techniques. In the intestines, 6735 and 12908 differently expressed genes (DEGs) were identified at 24 hpi and 48 hpi, respectively. Among 66 and 116 significantly enriched pathways, 15 and 21 pathways were involved in immune system or signal transduction at 24 hpi and 48 hpi, respectively. A number of genes involved in Toll-like receptor signaling pathway, RIG-I-like receptor signaling pathway, NOD-like receptor signaling pathway, T cell receptor signaling pathway, B cell receptor signaling pathway, Antigen processing and presentation, NF-kappa B signaling pathway and PI3K-Akt signaling pathway were significantly downregulated. In the skins, 3113 and 4467 DEGs were identified at 24 hpi and 48 hpi, respectively. Among 24 and 56 significantly enriched pathways, 4 and 13 pathways were involved in immune system or signal transduction at 24 hpi and 48 hpi, respectively. More immune-related signaling pathways including Leukocyte transendothelial migration, Cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, IL-17 signaling pathway, MAPK signaling pathway, TNF signaling pathway, Complement and coagulation cascades, Hematopoietic cell lineage and Jak-STAT signaling pathway were differently enriched for upregulated DEGs at 48 hpi, which were completely different from that in the intestines. Furthmore, comparative transcriptome analysis revealed that the downregulated 1618 genes and upregulated 1622 genes existed both at 24 hpi and 48 hpi for the intestine samples. In the skins, the downregulated 672 genes and upregulated 428 genes existed both at 24 hpi and 48 hpi. Three pathways related to immune processes were significantly enriched for downregulated DEGs both in the intestines and skins collected at 24 hpi and 48 hpi, which included Antigen processing and presentation, Intestinal immune network for IgA production and Hematopoietic cell lineage. Interaction network analysis of DEGs identified the main DEGs in the sub-network of complement and coagulation cascades both in the intestines and skins. Twenty of DEGs involved in complement and coagulation cascades were further validated by Real-time quantitative PCR. Altogether, the results obtained in this study will provide insight into the immune response of zebrafish against *S. agalactiae* XQ-1 infection in fatal conditions, and reveal the discrepant expression pattern of complement and coagulation cascades in the intestines and skins.

1. Introduction

Streptococcal bacteria are a versatile group of gram-positive bacteria, and cause invasive infections in humans and aquacultures. The most relevant streptococcal species include the human-specific *Streptococcus pneumoniae* and *Streptococcus pyogenes*, and the zoonotic *Streptococcus iniae* and *Streptococcus agalactiae* [1]. In mammals, Group A streptococcus (*S. pyogenes*) is a leading trigger of peripartum sepsis, cellulitis, bacteremia, necrotizing fasciitis and streptococcal toxic shock syndrome [2,3]. The pneumococcus (*S. pneumoniae*) is the major cause of community acquired pneumonia (CAP) and bacterial meningitis [4,5], and rare cause of infective endocarditis (IE), purulent

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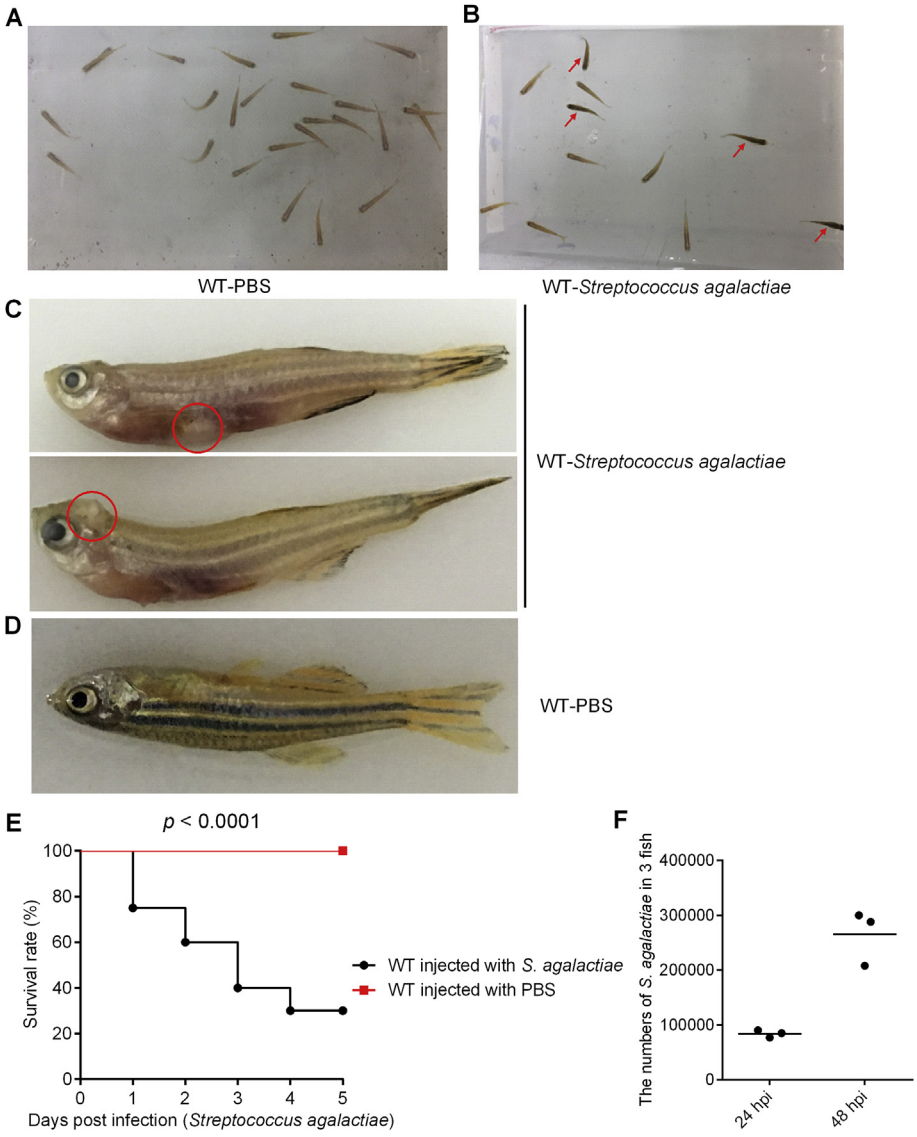

Fig. 1. *S. agalactiae* infection in zebrafish. (A) The wildtype zebrafish injected with PBS in the breeding container. (B) The wildtype zebrafish injected with *S. agalactiae* XQ-1 in the breeding container. The susceptible fish with darkened colouration were indicated in red arrows. (C) The dead zebrafish infected with *S. agalactiae* XQ-1. The visible cerebral edema or severe inflammation were circled in red. (D) The control zebrafish injected with PBS. (E) The survival curves of adult zebrafish injected with PBS or *S. agalactiae* XQ-1. Groups of 20 fish were used for survival assays. The survival curves were compared statistically significant difference using the Log-Rank Test. (F) Bacterial loads at 24 hpi and 48 hpi in the zebrafish injected with *S. agalactiae* XQ-1. The diffused liver and spleen were homogenated, and then serial dilutions of the homogenates were plated onto BHI agar. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Table 1
Ratio of mapping to zebrafish genome.

Sample	Total Read pairs	Total mapped reads	Clean base (bp)	Q20(%)	Q30(%)	GC(%)
WT-I24-1PBS	27,183,998	21,372,452(78.62%)	8,155,199,400	97.8;96.7	93.9;91.2	47.6;47.6
WT-I24-2PBS	27,086,476	20,695,946(76.41%)	8,125,942,800	97.7;96.2	93.8;90.1	47.3;47.3
WT-I48-1PBS	22,731,575	17,876,084(78.64%)	6,819,472,500	97.7;98.0	94.2;94.4	46.3;46.3
WT-I48-2PBS	23,437,590	18,547,869(79.14%)	7,031,277,000	97.7;97.8	94.2;94.0	47.1;47.1
WT-S24-1PBS	27,252,697	21,186,800(77.74%)	8,175,809,100	97.7;96.8	93.8;91.5	47.9;47.9
WT-S24-2PBS	22,285,415	17,325,402(77.74%)	6,685,624,500	97.6;96.9	93.7;91.8	48.1;48.1
WT-S48-1PBS	26,777,697	20,773,513(77.58%)	8,033,309,100	97.6;97.0	93.7;91.9	47.8;47.9
WT-S48-2PBS	28,236,029	21,836,462(77.34%)	8,470,808,700	97.7;96.1	93.9;89.8	47.7;47.7
WT-I24-1	26,818,144	19,947,494(74.38%)	8,045,443,200	98.2;97.8	95.3;94.0	47.5;47.4
WT-I24-2	29,809,873	22,297,581(74.80%)	8,942,961,900	98.2;97.7	95.3;93.7	47.6;47.5
WT-I48-1	27,734,787	21,358,439(77.01%)	8,320,436,100	98.2;97.8	95.3;93.9	48.3;48.3
WT-I48-2	22,044,226	17,049,803(77.34%)	6,613,267,800	98.2;97.9	95.3;94.1	48.4;48.4
WT-S24-1	23,954,705	17,436,028(72.79%)	7,186,411,500	98.0;97.6	95.0;93.8	48.7;48.7
WT-S24-2	20,964,362	15,423,517(73.57%)	6,289,308,600	97.9;97.7	94.8;93.9	48.7;48.6
WT-S48-1	24,736,167	18,172,123(73.46%)	7,420,850,100	98.1;97.3	95.1;92.8	48.1;48.0
WT-S48-2	24,232,795	17,645,946(72.82%)	7,269,838,500	98.0;97.5	95.0;93.2	47.9;47.8

pericarditis, septic arthritis, osteomyelitis, epidural and brain abscess [6]. The zoonotic streptococci can also cause opportunistic infections in humans. For instance, the Group B streptococcus (*S. agalactiae*) is an important cause of invasive infection in three populations including neonates and other infants, pregnant women and older adults with underlying chronic illness [6]. The most common clinical outcomes of a *S. agalactiae* infection in neonates are pneumonia, sepsis and meningitis [7].

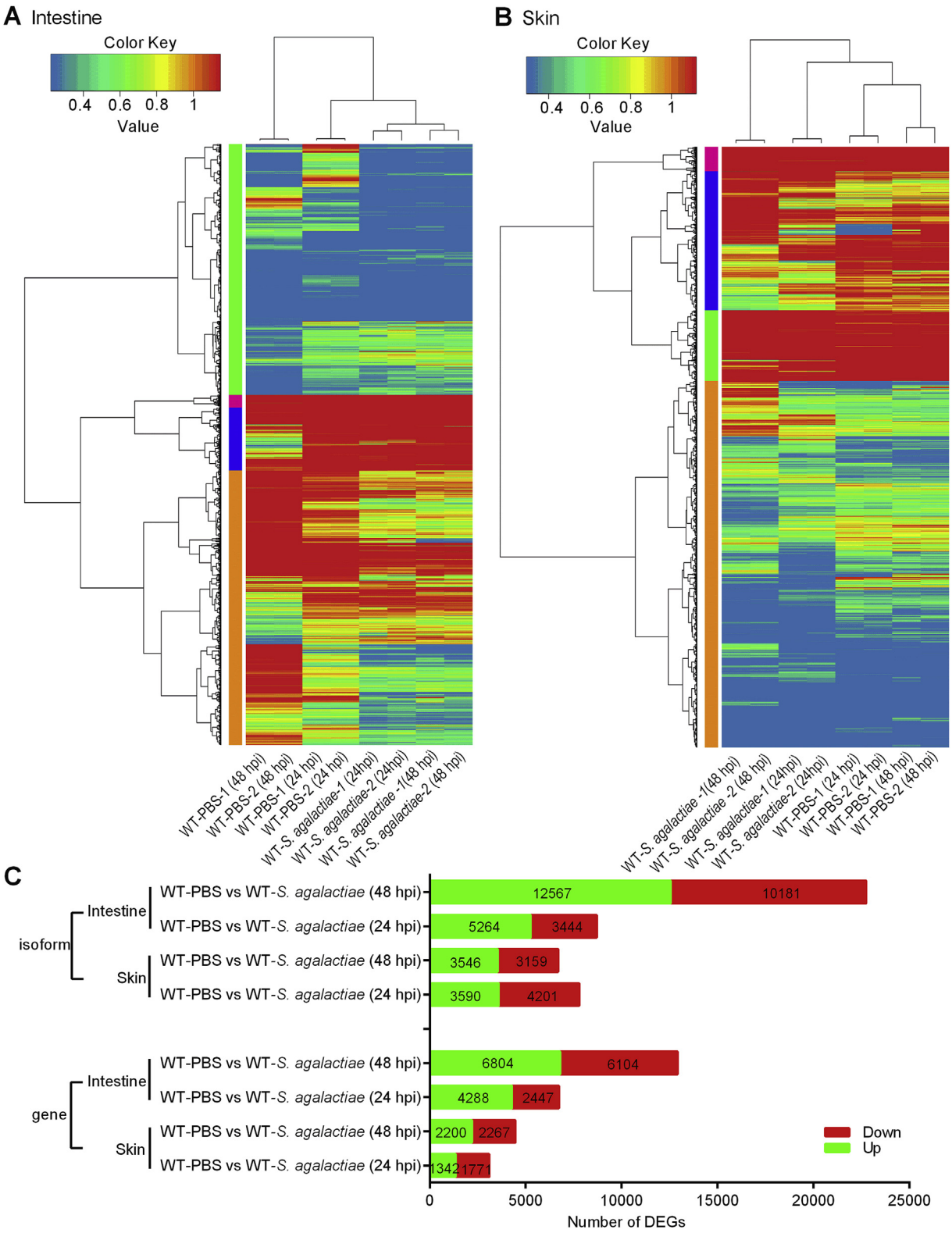

Fig. 2. RNAseq profiling in adult zebrafish injected with PBS and *S. agalactiae* XQ-1. (A) Heatmap analysis for transcriptomic data of intestine samples at 24 hpi and 48 hpi from the adult zebrafish injected with PBS and *S. agalactiae* XQ-1. (B) Heatmap analysis for transcriptomic data of skin samples at 24 hpi and 48 hpi from the adult zebrafish injected with PBS and *S. agalactiae* XQ-1. (C) Up- and down-regulated differentially expressed genes or isoforms from the intestine and skin samples collected at 24 hpi and 48 hpi. The up-regulated DEGs were highlighted in green, and down-regulated DEGs in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

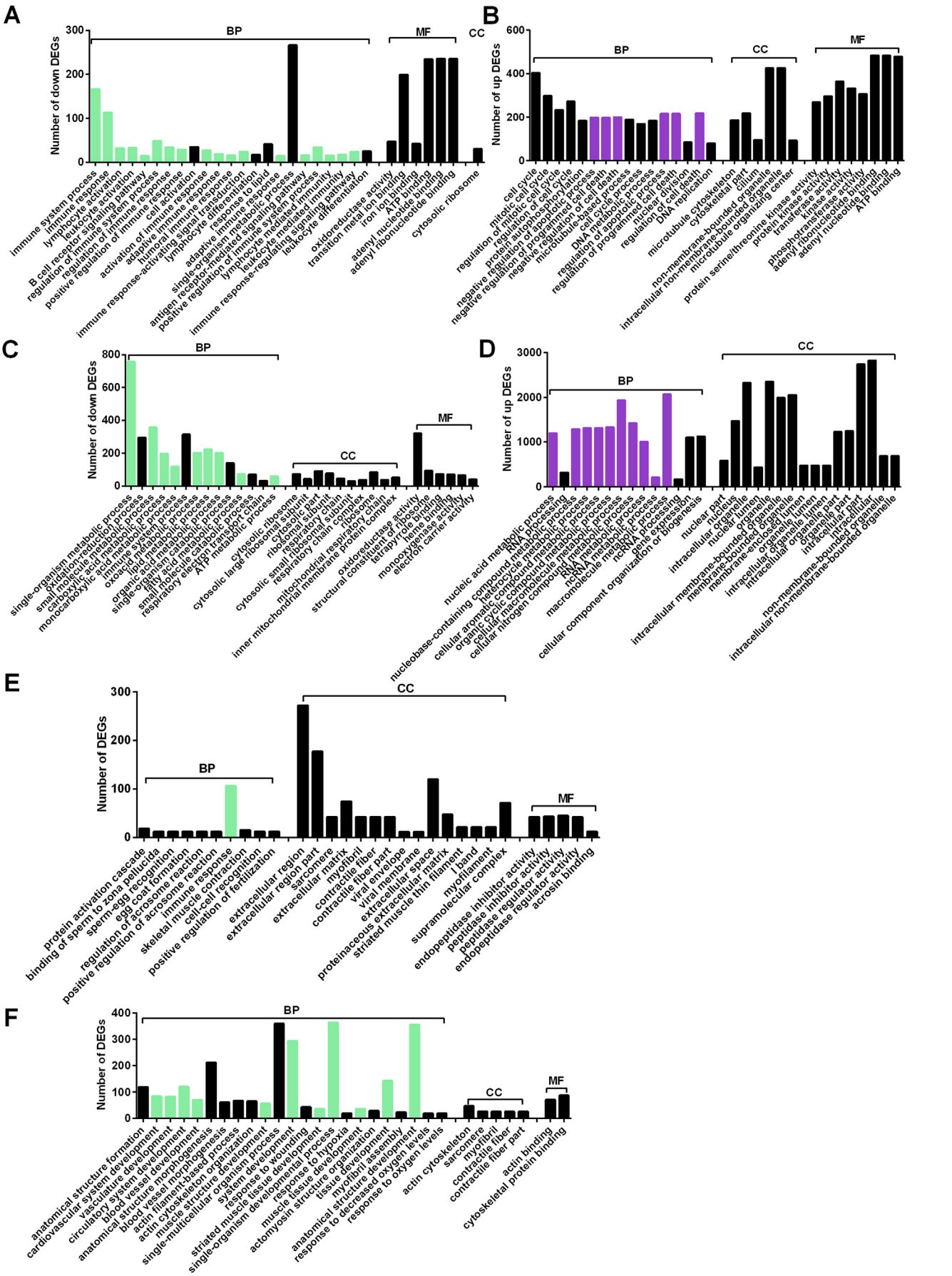

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Fig. 3. Gene ontology (GO) analysis of the deferentially expressed genes. (A) Top 30 GO terms were enriched as biochemical processes (BP), cellular components (CC), or molecular function (MF) for down-regulated DEGs in the intestines at 24 hpi. Those pathways involved in immune system process or immune response were highlighted in the color. (B) Top 30 GO terms were enriched as BP, CC or MF for up-regulated DEGs in the intestines at 24 hpi. Those pathways involved in the regulation of cell death or apoptotic process were highlighted in the color. (C) Top 30 GO terms were enriched as BP, CC or MF for down-regulated DEGs in the intestines at 48 hpi. Those pathways involved in metabolic process were highlighted in the color. (D) Top 30 GO terms were enriched as BP, CC or MF for up-regulated DEGs in the intestines at 48 hpi. Those pathways involved in metabolic process were highlighted in the color. (E) Top 30 GO terms were enriched as BP, CC or MF for DEGs in the skins at 24 hpi. The pathway involved in immune response was highlighted in the color. (F) Top 30 GO terms were enriched as BP, CC or MF for DEGs in the skins at 48 hpi. Those pathways involved in development biological process were highlighted in the color. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

In addition to infect mammals, *S. agalactiae* is also a major aquaculture pathogen infecting various saltwater and freshwater fish such as giant grouper [8], Javanese medaka [9], tilapia [10] and catfish [11]. Outbreaks of streptococcosis due to *S. agalactiae* infection have been described in wild fish and farmed fish [12–14]. Accordingly, different strategies used for the prevention of *S. agalactiae* infections have been developed, with 3 kinds of vaccines including formalin-killed vaccine, live attenuated vaccine and DNA vaccine [15–19]. Although many studies have characterized the molecular pathogenesis of *S. agalactiae* disease in mammals [20–22] and the immune responses to *S. agalactiae* infection in human, bovine and tilapia [23–27], the molecular mechanisms of host factors in response to *S. agalactiae* infection are limited.

With many similarities to mammalian systems, the zebrafish (*Danio rerio*) has a well-developed immune system, and is used as a valuable vertebrate model for the study of infectious diseases threatening both human health and aquaculture [28–30]. Multiple streptococcal infection models including *S. agalactiae* have already been established in zebrafish [1,31,32]. Several other studies used the natural interaction occurring between *S. agalactiae* and the zebrafish to understand *S. agalactiae* virulence and fish immunity [31,33,34]. However these studies regarding host factors in response to *S. agalactiae* infection were focused on the characterization of the expression of acute phase proteins and pro-inflammatory cytokines by suppression subtractive hybridization and/or qRT-PCR [31,35].

Despite transcriptome sequencing has been extensively utilized in fish immunology, only few transcriptomic studies were conducted in tilapia against *S. agalactiae* infection [23–25]. Up to date, no studies have systematically examined the innate immune response for the adult zebrafishwith the *S. agalactiae* infection through a high throughput method. Our preliminary experiments showed that the two time points at 24 h post-injection (hpi) and 48 hpi were suitable for samples collection, which could both reflect the bacterial proliferation and eliminate the stress response of small volume infection. Therefore, we examined transcriptional profiles of the intestines and skins at the two time points following experimental infection with *S. agalactiae*. Our data suggest that *S. agalactiae* infection influences multiple immune-related pathways which provide leads for functional studies of host-pathogen interactions. Furthermore, the present study for WT zebrafish-*S. agalactiae* infection will be extremely useful for better understanding of the molecular mechanism of *S. agalactiae* infection in susceptible and resistant populations generated by forward and reverse genetics approaches.

2. Materials and methods

2.1. Zebrafish

Wild-type AB/TU zebrafish were obtained from the China Zebrafish Resource Center (CZRC), and raised at 28 °C on a 12 h/12 h light/dark cycle in the flow-through system. Wild-type adult zebrafish (7 months old) were used throughout this study.

2.2. Bacterial strain

Streptococcus agalactiae XQ-1 strains were kindly given by Professor Ai-Hua Li from the same institute, and cultured in Brain Heart Infusion (BHI) medium at 37 °C.

2.3. Infection of zebrafish

Groups of zebrafish (30 fish per group) were injected intraperitoneally PBS or *S. agalactiae* strains (6.25×10^5 cfu/μl). For intraperitoneal injection, anesthetized fish were placed supine and supported by the moistened gauze. The needle was inserted up to the end of its bevel, and 10 μl of PBS or bacterial suspension was injected.

Groups of 20 fish were used for survival assays. The number of surviving zebrafish was counted daily for 5 days. GraphPad Prism 6 was used to generate survival curves, and the log-rank test was used to test differences in survival between the WT zebrafish injected with PBS and WT zebrafish injected with *S. agalactiae*.

Groups of 3 fish at each time point were used for measuring bacterial burden, transcriptome sequencing and qRT-PCR verification. The mixture of liver and spleen at 24 hpi and 48 hpi were rinsed and lysed in 500 μl of PBS. Serial dilutions of the homogenates were plated onto BHI agar, and CFU were enumerated after 12–16 h of incubation at 37 °C. The skins and intestines from groups of 3 fish at each time point were flash-frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.4. RNA isolation, cDNA library construction and RNA sequencing

Total RNA from skins and intestines collected at 24 hpi and 48 hpi was extracted using the TRIzol® Reagent (Invitrogen) following the manufacturer's protocol. RNA integrity was assessed using an Agilent 2100 bioanalyzer (Agilent, USA). Samples with RNA integrity numbers (RINs) ≥ 7.5 were subjected to cDNA library construction using TruseqTM RNA sample prep Kit (Illumina). Raw reads were produced by an Illumina Hiseq platform.

2.5. Transcriptome data analysis

The differentially expressed genes (DEGs) between the WT zebrafish injected with PBS and WT zebrafish injected with *S. agalactiae* were identified based on FDR (false discovery rate) < 0.05,$\log_2$FC (fold change (condition 2/condition 1) for a gene) > 1 or $\log_2$FC < −1. Gene Ontology (GO) enrichment analysis of the DEGs was implemented by the GOseq R package using Hyper-geometric distribution. Significantly enriched pathways of these DEGs were then determined by KEGG (Kyoto Encyclopedia of Genes and Genomes) database. Bonferroni correction was used to adjust *p*-values. GO terms and KEGG pathways with adjusted *p* value (Q-Value) < 0.05 were considered significantly enriched. Protein–protein interaction (PPI) analysis of DEGs was based on the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. Cytoscape software was applied to visualize the protein network.

2.6. qRT-PCR validation of DEGs

qRT-PCR analysis was performed to validate the DEGs involved in the complement and coagulation cascades pathway under the following conditions: 3 min at 95 °C, followed by 50 cycles of 15 s at 94 °C, 15 s at

Table 2
KEGG pathway enrichment analysis of intestine samples from WT-PBS and WT-*S. agalactiae* collected at 24 hpi.

Pathway Hierarchy2	KEGG Pathway	Pathway ID	Gene Number	Background number	Q-Value
Up DEGs					
Immune system	**Complement and coagulation cascades**	ko04610	63	132	1.43E-19
Replication and repair	DNA replication	ko03030	32	40	2.13E-19
Cell growth and death	Cell cycle - yeast	ko04111	44	91	4.29E-14
Cell growth and death	Cell cycle	ko04110	60	163	1.22E-12
Replication and repair	Fanconi anemia pathway	ko03460	30	59	2.28E-10
Replication and repair	Mismatch repair	ko03430	17	22	6.60E-10
Cell growth and death	Meiosis - yeast	ko04113	32	72	3.02E-09
Replication and repair	Homologous recombination	ko03440	21	37	1.64E-08
Replication and repair	Base excision repair	ko03410	20	48	2.68E-05
Replication and repair	Nucleotide excision repair	ko03420	19	44	2.68E-05
Nucleotide metabolism	Pyrimidine metabolism	ko00240	37	129	6.54E-05
Nucleotide metabolism	Purine metabolism	ko00230	52	236	2.67E-03
Cell growth and death	Oocyte meiosis	ko04114	33	148	3.26E-02
Down DEGs					
Translation	Ribosome	ko03010	35	134	1.07E-08
Immune system	**Antigen processing and presentation**	ko04612	28	102	1.16E-07
Immune system	**Th17 cell differentiation**	ko04659	36	158	1.16E-07
Immune system	**T cell receptor signaling pathway**	ko04660	33	144	3.61E-07
Signal transduction	**NF-kappa B signaling pathway**	ko04064	30	132	1.38E-06
Immune system	**Intestinal immune network for IgA production**	ko04672	22	78	1.38E-06
Immune system	**Th1 and Th2 cell differentiation**	ko04658	30	132	1.38E-06
Immune system	**Fc epsilon RI signaling pathway**	ko04664	24	104	1.64E-05
Immune system	**Hematopoietic cell lineage**	ko04640	27	126	1.64E-05
Immune system	**Natural killer cell mediated cytotoxicity**	ko04650	28	137	2.50E-05
Energy metabolism	Methane metabolism	ko00680	13	41	1.29E-04
Carbohydrate metabolism	Glycolysis/Gluconeogenesis	ko00010	19	85	2.95E-04
Signal transduction	FoxO signaling pathway	ko04068	33	202	3.71E-04
Signaling molecules and interaction	Cytokine-cytokine receptor interaction	ko04060	43	296	4.25E-04
Overview	Carbon metabolism	ko01200	25	143	1.03E-03
Environmental adaptation	Circadian rhythm	ko04710	13	51	1.18E-03
Signal transduction	HIF-1 signaling pathway	ko04066	25	146	1.29E-03
Overview	Degradation of aromatic compounds	ko01220	5	8	1.52E-03
Development	Osteoclast differentiation	ko04380	27	166	1.52E-03
Energy metabolism	Carbon fixation in photosynthetic organisms	ko00710	10	34	1.75E-03
Lipid metabolism	Linoleic acid metabolism	ko00591	10	35	2.18E-03
Endocrine system	PPAR signaling pathway	ko03320	18	94	2.27E-03
Endocrine system	Insulin signaling pathway	ko04910	29	196	3.84E-03
Immune system	**B cell receptor signaling pathway**	ko04662	21	127	5.31E-03
Metabolism of cofactors and vitamins	Retinol metabolism	ko00830	14	71	7.31E-03
Aging	Longevity regulating pathway - worm	ko04212	19	115	8.97E-03
Transport and catabolism	Lysosome	ko04142	26	180	9.29E-03
Lipid metabolism	Primary bile acid biosynthesis	ko00120	8	29	9.35E-03
Environmental adaptation	Circadian rhythm - fly	ko04711	6	17	9.41E-03
Signal transduction	AMPK signaling pathway	ko04152	26	183	1.07E-02
Immune system	**Fc gamma R-mediated phagocytosis**	ko04666	22	148	1.32E-02
Digestive system	Fat digestion and absorption	ko04975	11	54	1.57E-02
Sensory system	Inflammatory mediator regulation of TRP channels	ko04750	23	161	1.67E-02
Signal transduction	VEGF signaling pathway	ko04370	16	97	1.71E-02
Transport and catabolism	Autophagy - animal	ko04140	27	201	1.71E-02
Signal transduction	ErbB signaling pathway	ko04012	19	125	1.74E-02
Signal transduction	Ras signaling pathway	ko04014	41	346	1.74E-02
Carbohydrate metabolism	Fructose and mannose metabolism	ko00051	10	49	1.96E-02
Lipid metabolism	Arachidonic acid metabolism	ko00590	14	82	1.96E-02
Endocrine system	Ovarian Steroidogenesis	ko04913	23	165	1.96E-02
Signal transduction	**MAPK signaling pathway**	ko04010	44	385	2.20E-02
Signal transduction	Phospholipase D signaling pathway	ko04072	32	259	2.20E-02
Lipid metabolism	alpha-Linolenic acid metabolism	ko00592	7	28	2.20E-02
Xenobiotics biodegradation and metabolism	Naphthalene degradation	ko00626	3	5	2.20E-02
Aging	Longevity regulating pathway - multiple species	ko04213	16	102	2.20E-02
Immune system	**Toll-like receptor signaling pathway**	ko04620	19	131	2.36E-02
Signal transduction	**PI3K-Akt signaling pathway**	ko04151	55	513	2.65E-02
Folding, sorting and degradation	RNA degradation	ko03018	14	88	3.09E-02
Digestive system	Mineral absorption	ko04978	11	62	3.14E-02
Carbohydrate metabolism	Pyruvate metabolism	ko00620	10	54	3.24E-02
Carbohydrate metabolism	Pentose phosphate pathway	ko00030	7	32	4.24E-02
Signaling molecules and interaction	Cell adhesion molecules (CAMs)	ko04514	31	264	4.41E-02
Endocrine system	Adipocytokine signaling pathway	ko04920	16	113	4.98E-02

51–57 °C and 30 s at 72 °C. All reactions were performed in triplicate in a 96 well plate and the mean value recorded. Those DEGs for validation include A2M (XM_021466560.1, GeneID:100006993), C1S (NM_001114449.1, GeneID:793504), C3 (NM_001037236.2, GeneID:565074), C5 (XM_001919191.8, GeneID:565774), C8A (NM_001003496.1, GeneID:445102), C8B (NM_001256723.2, GeneID:793127), C8G (NM_200863.1, GeneID:393837), C9 (NM_001327926.1, GeneID:554141), CFH (XM_005161742.4,

Table 3
KEGG pathway enrichment analysis of intestine samples from WT-PBS and WT-*S. agalactiae* collected at 48 hpi.

Pathway Hierarchy2	KEGG Pathway	Pathway ID	Gene Number	Background number	Q-Value
Up DEGs					
Translation	RNA transport	ko03013	103	189	2.07E-14
Replication and repair	Fanconi anemia pathway	ko03460	45	59	1.43E-13
Translation	Ribosome biogenesis in eukaryotes	ko03008	54	85	3.79E-11
Replication and repair	Homologous recombination	ko03440	28	37	2.76E-08
Cell growth and death	Cell cycle - yeast	ko04111	50	91	2.09E-07
Transcription	Spliceosome	ko03040	72	149	2.09E-07
Folding, sorting and degradation	Ubiquitin mediated proteolysis	ko04120	77	167	6.82E-07
Replication and repair	DNA replication	ko03030	27	40	1.64E-06
Replication and repair	Mismatch repair	ko03430	18	22	2.22E-06
Folding, sorting and degradation	RNA degradation	ko03018	45	88	1.21E-05
Cell growth and death	Cell cycle	ko04110	71	163	2.12E-05
Replication and repair	Nucleotide excision repair	ko03420	27	44	2.12E-05
Nucleotide metabolism	Pyrimidine metabolism	ko00240	59	129	2.31E-05
Endocrine system	Progesterone-mediated oocyte maturation	ko04914	53	120	2.59E-04
Cell growth and death	Meiosis - yeast	ko04113	35	72	6.15E-04
Transcription	RNA polymerase	ko03020	20	34	9.49E-04
Translation	mRNA surveillance pathway	ko03015	41	96	4.72E-03
Transcription	Basal transcription factors	ko03022	23	46	6.68E-03
Replication and repair	Base excision repair	ko03410	22	48	3.57E-02
Cell growth and death	Oocyte meiosis	ko04114	54	148	4.55E-02
Down DEGs					
Translation	Ribosome	ko03010	89	134	1.15E-29
Xenobiotics biodegradation and metabolism	Metabolism of xenobiotics by cytochrome P450	ko00980	40	59	1.71E-13
Xenobiotics biodegradation and metabolism	Drug metabolism - cytochrome P450	ko00982	38	56	5.64E-13
Energy metabolism	Oxidative phosphorylation	ko00190	68	147	1.68E-11
Transport and catabolism	Peroxisome	ko04146	54	106	3.08E-11
Digestive system	Fat digestion and absorption	ko04975	34	54	1.96E-10
Signaling molecules and interaction	**Cytokine-cytokine receptor interaction**	ko04060	105	296	4.40E-09
Immune system	**Th1 and Th2 cell differentiation**	ko04658	57	132	1.98E-08
Metabolism of cofactors and vitamins	Retinol metabolism	ko00830	37	71	3.33E-08
Immune system	**Th17 cell differentiation**	ko04659	64	158	3.50E-08
Endocrine system	PPAR signaling pathway	ko03320	44	94	6.27E-08
Lipid metabolism	Glycerolipid metabolism	ko00561	38	76	6.89E-08
Lipid metabolism	Steroid hormone biosynthesis	ko00140	30	57	6.43E-07
Lipid metabolism	Fatty acid degradation	ko00071	29	57	2.68E-06
Carbohydrate metabolism	Pentose and glucuronate interconversions	ko00040	22	38	4.27E-06
Carbohydrate metabolism	Ascorbate and aldarate metabolism	ko00053	21	36	6.34E-06
Amino acid metabolism	Arginine and proline metabolism	ko00330	34	79	2.95E-05
Signal transduction	**TNF signaling pathway**	ko04668	54	152	5.02E-05
Lipid metabolism	Glycerophospholipid metabolism	ko00564	48	130	5.02E-05
Signal transduction	**NF-kappa B signaling pathway**	ko04064	48	132	7.79E-05
Metabolism of other amino acids	Glutathione metabolism	ko00480	32	76	8.39E-05
Lipid metabolism	Steroid biosynthesis	ko00100	14	22	1.05E-04
Digestive system	Vitamin digestion and absorption	ko04977	21	42	1.24E-04
Immune system	**Antigen processing and presentation**	ko04612	39	102	1.24E-04
Lipid metabolism	Arachidonic acid metabolism	ko00590	33	82	1.49E-04
Development	Osteoclast differentiation	ko04380	56	166	1.49E-04
Digestive system	Protein digestion and absorption	ko04974	52	151	1.49E-04
Immune system	**T cell receptor signaling pathway**	ko04660	50	144	1.57E-04
Amino acid metabolism	Tryptophan metabolism	ko00380	25	56	1.84E-04
Cell growth and death	Necroptosis	ko04217	73	236	2.17E-04
Lipid metabolism	Primary bile acid biosynthesis	ko00120	16	29	2.18E-04
Lipid metabolism	Linoleic acid metabolism	ko00591	18	35	2.36E-04
Overview	Carbon metabolism	ko01200	49	143	2.41E-04
Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	ko00520	27	64	2.58E-04
Digestive system	Bile secretion	ko04976	62	195	3.01E-04
Amino acid metabolism	Glycine, serine and threonine metabolism	ko00260	22	49	4.06E-04
Carbohydrate metabolism	Fructose and mannose metabolism	ko00051	22	49	4.06E-04
Immune system	**Toll-like receptor signaling pathway**	ko04620	45	131	4.06E-04
Lipid metabolism	Sphingolipid metabolism	ko00600	29	73	4.44E-04
Lipid metabolism	alpha-Linolenic acid metabolism	ko00592	15	28	4.82E-04
Immune system	**RIG-I-like receptor signaling pathway**	ko04622	33	88	5.29E-04
Immune system	**Natural killer cell mediated cytotoxicity**	ko04650	46	137	5.74E-04
Circulatory system	Cardiac muscle contraction	ko04260	43	127	7.58E-04
Lipid metabolism	Ether lipid metabolism	ko00565	25	62	9.37E-04
Endocrine system	Aldosterone synthesis and secretion	ko04925	64	214	1.32E-03
Xenobiotics biodegradation and metabolism	Drug metabolism - other enzymes	ko00983	24	60	1.38E-03
Digestive system	Pancreatic secretion	ko04972	53	170	1.38E-03
Immune system	**Fc epsilon RI signaling pathway**	ko04664	36	104	1.45E-03
Signal transduction	**Jak-STAT signaling pathway**	ko04630	55	179	1.51E-03
Carbohydrate metabolism	Galactose metabolism	ko00052	17	37	1.51E-03
Endocrine system	Adipocytokine signaling pathway	ko04920	38	113	1.81E-03
Environmental adaptation	Circadian rhythm	ko04710	21	51	1.88E-03

(*continued on next page*)

Table 3 (*continued*)

Pathway Hierarchy2	KEGG Pathway	Pathway ID	Gene Number	Background number	Q-Value
Carbohydrate metabolism	Glycolysis/Gluconeogenesis	ko00010	30	85	2.82E-03
Immune system	**NOD-like receptor signaling pathway**	ko04621	65	225	2.82E-03
Immune system	**Intestinal immune network for IgA production**	ko04672	28	78	3.07E-03
Overview	Fatty acid metabolism	ko01212	26	71	3.29E-03
Sensory system	Inflammatory mediator regulation of TRP channels	ko04750	49	161	3.53E-03
Signal transduction	**MAPK signaling pathway - plant**	ko04016	10	18	3.87E-03
Cell growth and death	Ferroptosis	ko04216	23	61	3.93E-03
Endocrine system	Renin-angiotensin system	ko04614	12	24	3.93E-03
Immune system	**Hematopoietic cell lineage**	ko04640	40	126	3.98E-03
Immune system	**B cell receptor signaling pathway**	ko04662	40	127	4.65E-03
Digestive system	Mineral absorption	ko04978	23	62	4.78E-03
Lipid metabolism	Biosynthesis of unsaturated fatty acids	ko01040	14	31	4.91E-03
Overview	Degradation of aromatic compounds	ko01220	6	8	4.91E-03
Biosynthesis of other secondary metabolites	Penicillin and cephalosporin biosynthesis	ko00311	4	4	6.34E-03
Metabolism of other amino acids	D-Arginine and D-ornithine metabolism	ko00472	4	4	6.34E-03
Carbohydrate metabolism	Citrate cycle (TCA cycle)	ko00020	16	39	7.34E-03
Metabolism of other amino acids	beta-Alanine metabolism	ko00410	16	39	7.34E-03
Amino acid metabolism	Histidine metabolism	ko00340	13	29	7.41E-03
Signal transduction	Phosphatidylinositol signaling system	ko04070	41	139	1.39E-02
Immune system	**Chemokine signaling pathway**	ko04062	76	290	1.47E-02
Immune system	**Fc gamma R-mediated phagocytosis**	ko04666	43	148	1.47E-02
Immune system	**IL-17 signaling pathway**	ko04657	35	115	1.47E-02
Signal transduction	**PI3K-Akt signaling pathway**	ko04151	125	513	1.76E-02
Glycan biosynthesis and metabolism	Mucin type O-glycan biosynthesis	ko00512	17	46	1.79E-02
Digestive system	Carbohydrate digestion and absorption	ko04973	20	58	2.13E-02
Xenobiotics biodegradation and metabolism	Chloroalkane and chloroalkene degradation	ko00625	7	13	2.28E-02
Xenobiotics biodegradation and metabolism	Naphthalene degradation	ko00626	4	5	2.28E-02
Amino acid metabolism	Tyrosine metabolism	ko00350	15	40	2.38E-02
Glycan biosynthesis and metabolism	Other glycan degradation	ko00511	11	26	2.43E-02
Carbohydrate metabolism	Inositol phosphate metabolism	ko00562	33	111	2.49E-02
Endocrine system	Glucagon signaling pathway	ko04922	42	149	2.57E-02
Cell growth and death	Apoptosis	ko04210	71	276	2.72E-02
Energy metabolism	Methane metabolism	ko00680	15	41	2.91E-02
Carbohydrate metabolism	Starch and sucrose metabolism	ko00500	16	45	3.01E-02
Lipid metabolism	Fatty acid elongation	ko00062	16	45	3.01E-02
Endocrine system	Renin secretion	ko04924	34	117	3.01E-02
Xenobiotics biodegradation and metabolism	Caprolactam degradation	ko00930	5	8	3.08E-02
Environmental adaptation	Circadian rhythm - fly	ko04711	8	17	3.11E-02
Energy metabolism	Sulfur metabolism	ko00920	6	11	3.34E-02
Sensory system	Phototransduction - fly	ko04745	16	46	3.62E-02
Carbohydrate metabolism	Pyruvate metabolism	ko00620	18	54	3.83E-02
Membrane transport	ABC transporters	ko02010	19	58	3.87E-02
Transport and catabolism	Lysosome	ko04142	48	180	4.03E-02
Signal transduction	cGMP - PKG signaling pathway	ko04022	73	294	5.00E-02

GeneID:101884308), f7i (NM_173228.1, GeneID:282671), F13A1L (XM_681557.8 GeneID:558353), FGA (NM_001194989.1, GeneID:378986), FGB (NM_212774.1, GeneID:337315), FGG (NM_213054.1, GeneID:406327), MBL (XM_001341879.6, GeneID:100007982), PLG (NM_201472.2, GeneID:322691), VTN (NM_001139461.1 GeneID:403026), SERPINF2 (XM_683705.7, GeneID:560308), SERPINC1 (NM_182863.1, GeneID:321545) and SERPINE1 (NM_001114559.1, GeneID:100136840). The housekeeping gene GAPDH was used for normalizing cDNA amounts. The primers specific for the interested DEGs were listed in Supplementary Table 1.

2.7. Statistical analysis

Expression data by qRT-PCR are presented as means and standard error of mean (SEM). Two-tailed Student's t-test were used to compare means and SEM between groups. All data are representative of three biologic replications. The level of significance is shown as follows: *, $p < 0.05$; **, $p < 0.01$. Significance testing in the cumulative survival analysis used Log Rank test.

3. Results

3.1. S. agalactiae infection in zebrafish

Previous study showed that infection with 10^6 cfu *S. agalactiae* FIM314 resulted in the death of all zebrafish for both intraperitoneal and intramuscular routes within 3–4 days [31]. In order to investigate the virulence capacity of *S. agalactiae* XQ-1 strain, group of 20 adult fish were challenged with 6.25×10^6 cfu per fish. Compared with control zebrafish injected with PBS (Fig. 1A), susceptible fish showed erratic swimming behavior or swimming abnormally near the surface of the water with darkened colouration (Fig. 1B). The dead zebrafish infected with *S. agalactiae* XQ-1 appeared visible cerebral edema or severe inflammation (Fig. 1C). Control fish exhibited no abnormal behaviors, symptom-free and no death throughout these experiments (Fig. 1A and Fig. 1D). Intraperitoneal injections caused mortalities from 1 to 4 days post-injection (dpi), with the survival rate of 75% at 1 dpi, 60% at 2 dpi, 40% at 3 dpi, 30% at 4 and 5 dpi (Fig. 1E). Internal organs of diffused liver and spleen were harvested from infected zebrafish and analyzed for *S. agalactiae* XQ-1 by plating on BHI agar. Viable *S. agalactiae* XQ-1 organisms ($> 10^5$ CFU) could be detected in internal organs (Fig. 1F). A PCR and sequence analysis of selected colonies isolated from infected zebrafish confirmed these as *S. agalactiae* XQ-1.

Table 4
KEGG pathway enrichment analysis of skin samples from WT-PBS and WT-*S. agalactiae* collected at 24 hpi.

Pathway Hierarchy2	KEGG Pathway	Pathway ID	Gene Number	Background number	Q-Value
Up DEGs					
Immune system	**IL-17 signaling pathway**	ko04657	15	115	0.010
Circulatory system	Cardiac muscle contraction	ko04260	15	127	0.016
Development	Osteoclast differentiation	ko04380	17	166	0.023
Down DEGs					
Immune system	**Complement and coagulation cascades**	ko04610	43	132	1.80E-20
Immune system	**Antigen processing and presentation**	ko04612	21	102	9.25E-06
Xenobiotics biodegradation and metabolism	Drug metabolism - cytochrome P450	ko00982	14	56	7.82E-05
Carbohydrate metabolism	Glycolysis/Gluconeogenesis	ko00010	16	85	5.18E-04
Xenobiotics biodegradation and metabolism	Metabolism of xenobiotics by cytochrome P450	ko00980	13	59	5.18E-04
Digestive system	Protein digestion and absorption	ko04974	22	151	6.69E-04
Transport and catabolism	Phagosome	ko04145	27	231	3.23E-03
Lipid metabolism	Steroid biosynthesis	ko00100	7	22	3.23E-03
Cellular community - eukaryotes	Focal adhesion	ko04510	33	334	1.28E-02
Signaling molecules and interaction	ECM-receptor interaction	ko04512	16	118	1.28E-02
Metabolism of other amino acids	Glutathione metabolism	ko00480	12	76	1.45E-02
Nervous system	Serotonergic synapse	ko04726	21	184	1.67E-02
Energy metabolism	Methane metabolism	ko00680	8	41	2.39E-02
Signal transduction	**PI3K-Akt signaling pathway**	ko04151	43	513	3.36E-02
Lipid metabolism	Linoleic acid metabolism	ko00591	7	35	3.36E-02
Digestive system	Fat digestion and absorption	ko04975	9	54	3.36E-02
Amino acid metabolism	Arginine and proline metabolism	ko00330	11	79	3.73E-02
Amino acid metabolism	Phenylalanine metabolism	ko00360	5	19	3.73E-02
Immune system	Hematopoietic cell lineage	ko04640	15	126	3.73E-02
Nervous system	Glutamatergic synapse	ko04724	21	204	3.73E-02
Digestive system	Pancreatic secretion	ko04972	18	170	4.96E-02

3.2. RNA sequencing results

To investigate the gene changes related to *S. agalactiae* XQ-1 infection, total RNA was extracted from skin and intestine samples from adult zebrafish injected with PBS or *S. agalactiae* XQ-1. Quality control analyses and reads mapped information are shown in Table 1. Comparing with the reference sequence of the genome (http://asia.ensembl.org/Danio_rerio/Info/Index), more than 70% of total read pairs were mapped on the zebrafish genome (Table 1).

3.3. Differential gene expression analysis

The transcriptomes of intestines and skins isolated from adult zebrafish at 24 hpi and 48 hpi with or without *S. agalactiae* XQ-1 infection were obtained using RNASeq analysis (Fig. 2A and B). Along with the known transcripts, RNASeq analysis detected also many variants of known transcripts and novel transcripts. Comparing the intestine profiles from the zebrafish injected with PBS and *S. agalactiae* XQ-1, 6735 and 12908 DEGs were revealed at 24 hpi and 48 hpi, respectively. Among these genes, 4288 were up-regulated (3918 known transcripts) while 2447 were down-regulated (2109 known transcripts) at 24 hpi, 6804 were up-regulated (6493 known transcripts) while 6104 were down-regulated (5421 known transcripts) at 48 hpi. Differential transcript/isoform expression analyses revealed 8708 and 22748 differentially expressed isoforms (DEIs) at 24 hpi and 48 hpi, respectively. Among these isoforms, 5264 were up-regulated (3333 known transcripts) while 3444 were down-regulated (1821 known transcripts) at 24 hpi, 12567 were up-regulated (8460 known transcripts) while 10181 were down-regulated (5737 known transcripts) at 48 hpi (Fig. 2C).

Comparing the skin profiles from the zebrafish injected with PBS and *S. agalactiae* XQ-1, 3113 and 4467 DEGs were revealed at 24 hpi and 48 hpi, respectively. Among these genes, 1342 were up-regulated (1140 known transcripts) while 1771 were down-regulated (1607 known transcripts) at 24 hpi, 2200 were up-regulated (1961 known transcripts) while 2267 were down-regulated (2105 known transcripts) at 48 hpi. 7791 and 6705 DEIs were found at 24 hpi and 48 hpi, respectively. Among these isoforms, 3590 were up-regulated (1849 known transcripts) while 4201 were down-regulated (2515 known transcripts) at 24 hpi, 3546 were up-regulated (1992 known transcripts) while 3159 were down-regulated (1986 known transcripts) at 48 hpi (Fig. 2C).

3.4. GO enrichment analysis of DEGs

To explore the biological functions of these DEGs, GO analysis was performed based on the GO annotation terms. Enriched GO terms were classified to biological process (BP) class, cellular component (CC) class and molecular function (MF) class. For the intestines at 24 hpi, 18 out of the top 30 GO terms differently enriched downregulated genes were involved in immune system process or immune response (Fig. 3A), and 6 out of the top 30 GO terms differently enriched upregulated genes belonged to regulation of cell death or apoptotic process (Fig. 3B). For the intestines at 48 hpi, 9 and 10 out of the top 30 GO terms differently enriched downregulated and upregulated genes were involved in metabolic process (Fig. 3C and D). For the skins at 24 hpi, only 1 out of the top 30 GO terms differently enriched genes were involved in immune response, however the numbers of DEGs involved in immune response were the highest according to BP class (Fig. 3E). For the skins at 48 hpi, 11 out of the top 30 GO terms differently enriched genes were involved in development biological process (Fig. 3F).

3.5. KEGG pathway enrichment analysis

KEGG analysis significantly identified multiple immune-related pathways. Among 66 pathways for DEGs in the intestines at 24 hpi, 15 pathways involved in immune system or signal transduction were identified, which included Complement and coagulation cascades, Antigen processing and presentation, Th17 cell differentiation, T cell receptor signaling pathway, NF-kappa B signaling pathway, Intestinal immune network for IgA production, Th1 and Th2 cell differentiation, Fc epsilon RI signaling pathway, Hematopoietic cell lineage, Natural killer cell mediated cytotoxicity, B cell receptor signaling pathway, Fc gamma R-mediated phagocytosis, MAPK signaling pathway, Toll-like receptor signaling pathway and PI3K-Akt signaling pathway (Table 2). Among 116 pathways for DEGs in the intestines at 48 hpi, 21 pathways involved in immune system or signal transduction were identified,

Table 5
KEGG pathway enrichment analysis of skin samples from WT-PBS and WT-*S. agalactiae* collected at 48 hpi.

Pathway Hierarchy2	KEGG Pathway	Pathway ID	Gene Number	Background number	Q-Value
Up DEGs					
Cellular community - eukaryotes	Focal adhesion	ko04510	59	334	1.98E-07
Immune system	**Leukocyte transendothelial migration**	ko04670	42	228	8.01E-06
Signaling molecules and interaction	**Cytokine-cytokine receptor interaction**	ko04060	49	296	1.45E-05
Cell motility	Regulation of actin cytoskeleton	ko04810	54	359	6.15E-05
Signal transduction	**PI3K-Akt signaling pathway**	ko04151	69	513	1.11E-04
Cellular community - eukaryotes	Tight junction	ko04530	44	296	5.45E-04
Carbohydrate metabolism	Glycolysis/Gluconeogenesis	ko00010	19	85	5.51E-04
Immune system	**IL-17 signaling pathway**	ko04657	22	115	1.43E-03
Signaling molecules and interaction	ECM-receptor interaction	ko04512	22	118	1.72E-03
Signal transduction	**MAPK signaling pathway**	ko04010	51	385	1.72E-03
Circulatory system	Cardiac muscle contraction	ko04260	23	127	1.72E-03
Signal transduction	**TNF signaling pathway**	ko04668	25	152	3.93E-03
Transport and catabolism	Phagosome	ko04145	33	231	5.65E-03
Development	Osteoclast differentiation	ko04380	26	166	5.65E-03
Immune system	**Complement and coagulation cascades**	ko04610	22	132	6.13E-03
Immune system	**Hematopoietic cell lineage**	ko04640	21	126	7.72E-03
Signal transduction	**Jak-STAT signaling pathway**	ko04630	26	179	1.49E-02
Membrane transport	ABC transporters	ko02010	12	58	1.58E-02
Excretory system	Collecting duct acid secretion	ko04966	8	30	1.77E-02
Cellular community - prokaryotes	Biofilm formation - *Escherichia coli*	ko02026	3	4	1.97E-02
Digestive system	Bile secretion	ko04976	27	195	2.01E-02
Cellular community - eukaryotes	Adherens junction	ko04520	23	159	2.14E-02
Signal transduction	Rap1 signaling pathway	ko04015	45	381	2.14E-02
Carbohydrate metabolism	Pyruvate metabolism	ko00620	11	54	2.14E-02
Signal transduction	VEGF signaling pathway	ko04370	16	97	2.44E-02
Overview	Carbon metabolism	ko01200	21	143	2.46E-02
Down DEGs					
Immune system	**Complement and coagulation cascades**	ko04610	38	132	3.58E-11
Xenobiotics biodegradation and metabolism	Metabolism of xenobiotics by cytochrome P450	ko00980	21	59	6.87E-08
Xenobiotics biodegradation and metabolism	Drug metabolism - cytochrome P450	ko00982	18	56	4.91E-06
Immune system	**Antigen processing and presentation**	ko04612	23	102	7.61E-05
Immune system	**Hematopoietic cell lineage**	ko04640	26	126	7.65E-05
Metabolism of cofactors and vitamins	Retinol metabolism	ko00830	18	71	1.24E-04
Digestive system	Protein digestion and absorption	ko04974	28	151	2.00E-04
Lipid metabolism	Steroid hormone biosynthesis	ko00140	15	57	3.79E-04
Lipid metabolism	Steroid biosynthesis	ko00100	9	22	3.90E-04
Xenobiotics biodegradation and metabolism	Bisphenol degradation	ko00363	4	4	7.87E-04
Immune system	**Intestinal immune network for IgA production**	ko04672	17	78	1.07E-03
Signaling molecules and interaction	Cell adhesion molecules (CAMs)	ko04514	37	264	2.83E-03
Metabolism of other amino acids	Glutathione metabolism	ko00480	15	76	7.95E-03
Xenobiotics biodegradation and metabolism	Aminobenzoate degradation	ko00627	6	15	8.40E-03
Digestive system	Fat digestion and absorption	ko04975	12	54	8.40E-03
Amino acid metabolism	Tyrosine metabolism	ko00350	10	40	8.55E-03
Metabolism of cofactors and vitamins	One carbon pool by folate	ko00670	7	21	8.56E-03
Amino acid metabolism	Alanine, aspartate and glutamate metabolism	ko00250	11	48	9.04E-03
Amino acid metabolism	Tryptophan metabolism	ko00380	12	56	9.41E-03
Digestive system	Vitamin digestion and absorption	ko04977	10	42	1.04E-02
Excretory system	Proximal tubule bicarbonate reclamation	ko04964	11	50	1.12E-02
Carbohydrate metabolism	Ascorbate and aldarate metabolism	ko00053	9	36	1.17E-02
Replication and repair	DNA replication	ko03030	9	40	2.50E-02
Amino acid metabolism	Arginine biosynthesis	ko00220	8	33	2.53E-02
Lipid metabolism	Arachidonic acid metabolism	ko00590	14	82	2.76E-02
Metabolism of cofactors and vitamins	Folate biosynthesis	ko00790	7	27	2.89E-02
Immune system	**Th1 and Th2 cell differentiation**	ko04658	19	132	3.81E-02
Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	ko00630	8	36	3.88E-02
Amino acid metabolism	Arginine and proline metabolism	ko00330	13	79	4.45E-02
Amino acid metabolism	Cysteine and methionine metabolism	ko00270	10	53	4.45E-02

which included Cytokine-cytokine receptor interaction, Th1 and Th2 cell differentiation, Th17 cell differentiation, TNF signaling pathway, NF-kappa B signaling pathway, Antigen processing and presentation, T cell receptor signaling pathway, Toll-like receptor signaling pathway, RIG-I-like receptor signaling pathway, Natural killer cell mediated cytotoxicity, Fc epsilon RI signaling pathway, Jak-STAT signaling pathway, NOD-like receptor signaling pathway, Intestinal immune network for IgA production, MAPK signaling pathway, Hematopoietic cell lineage, B cell receptor signaling pathway, Chemokine signaling pathway, Fc gamma R-mediated phagocytosis, IL-17 signaling pathway and PI3K-Akt signaling pathway (Table 3).

Among 24 pathways for DEGs in the skins at 24 hpi, 4 pathways involved in immune system or signal transduction were identified, which included IL-17 signaling pathway, Complement and coagulation cascades, Antigen processing and presentation and PI3K-Akt signaling pathway (Table 4). Among 56 pathways for DEGs in the skins at 48 hpi, 13 pathways involved in immune system or signal transduction were identified, which included Leukocyte transendothelial migration, Cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, IL-17 signaling pathway, MAPK signaling pathway, TNF signaling pathway, Complement and coagulation cascades, Hematopoietic cell lineage, Jak-STAT signaling pathway, Antigen processing and presentation, Hematopoietic cell lineage, Intestinal immune network for IgA production and Th1 and Th2 cell differentiation (Table 5).

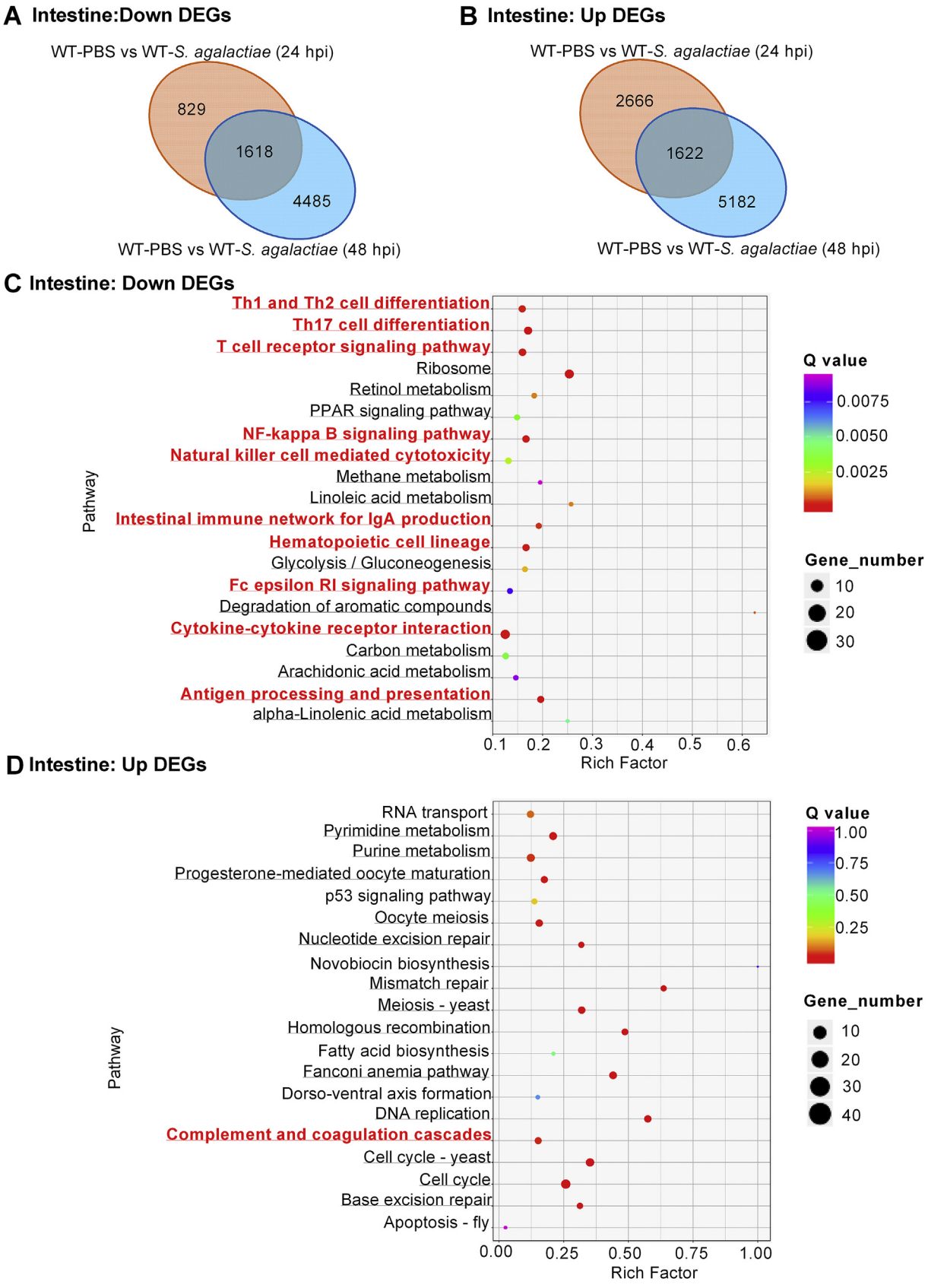
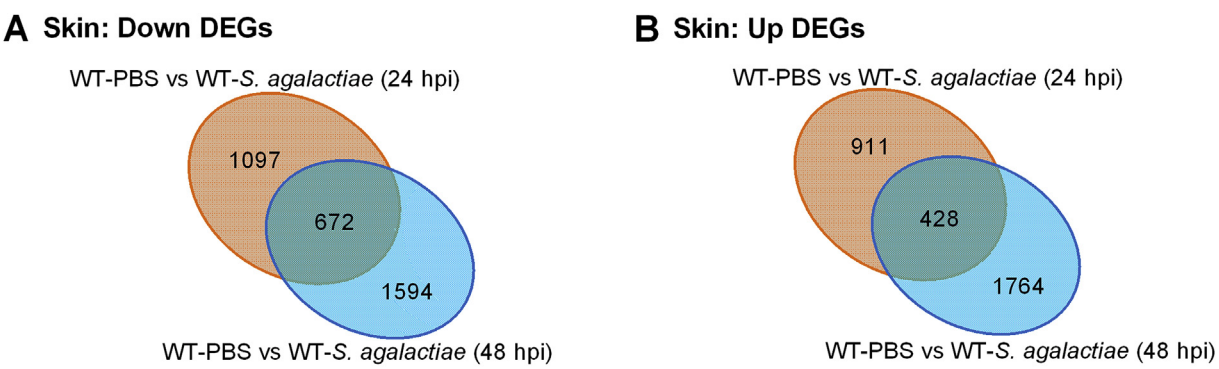
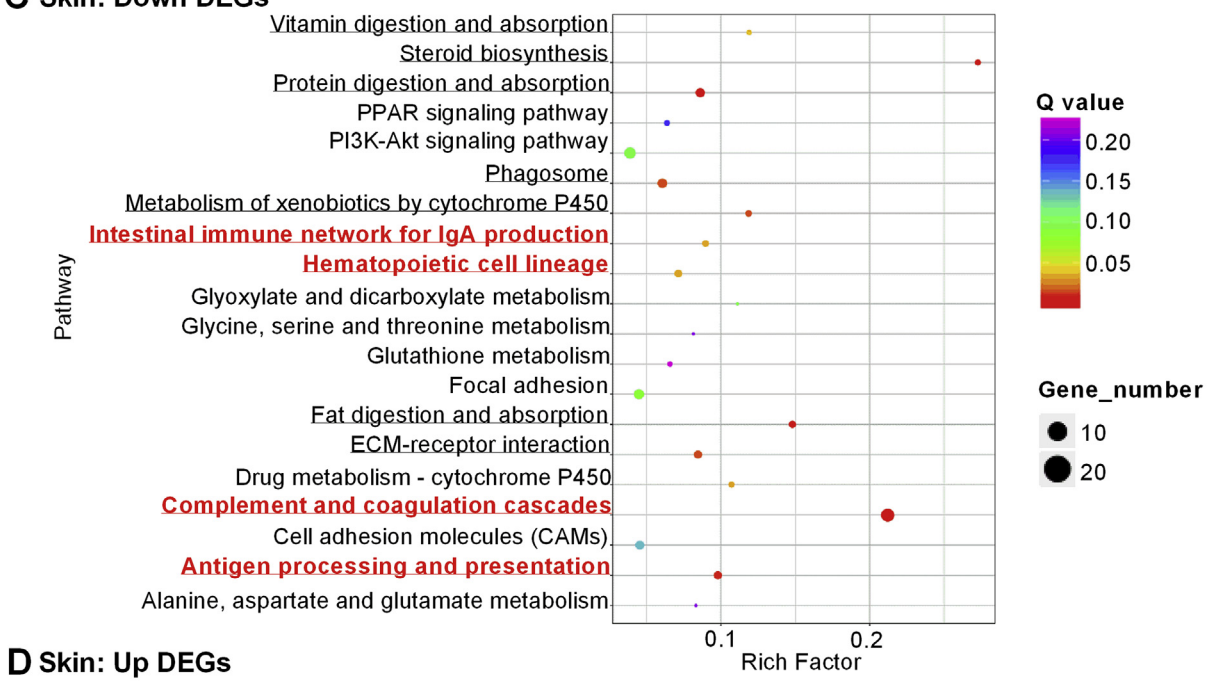
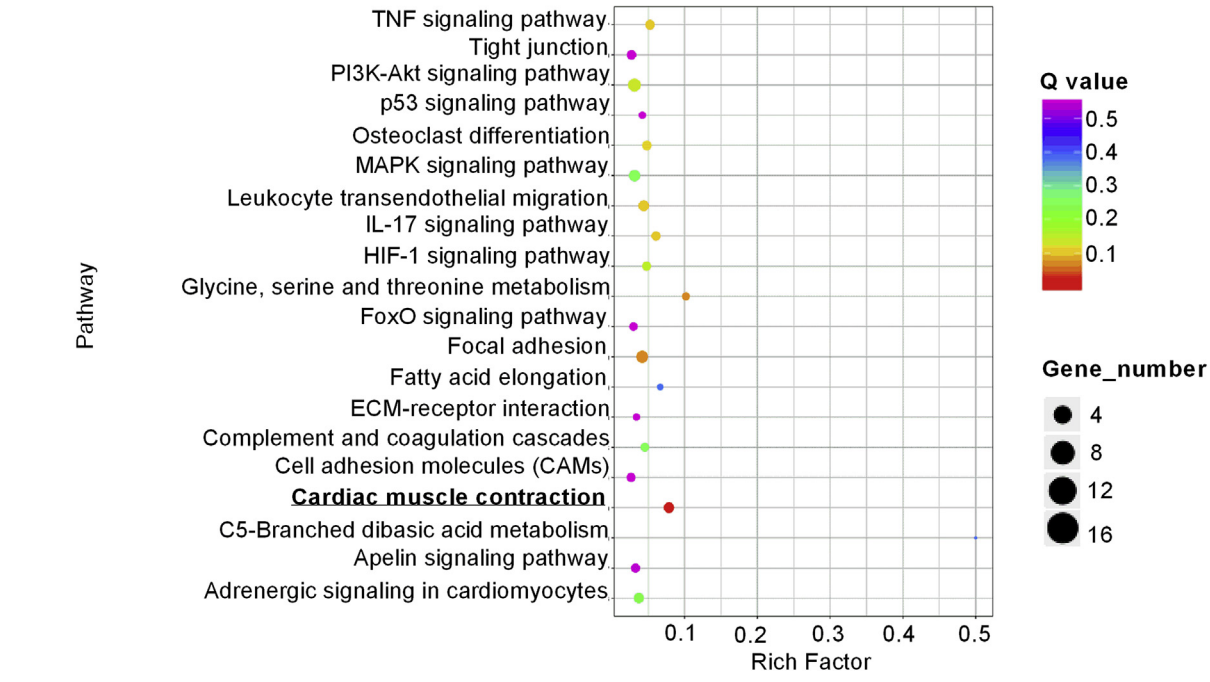


Fig. 4. Comparative transcriptome analysis of the common DEGs in the intestines. (A) Venn diagrams showing overlaps of down-regulated genes in intestine samples collected at 24 hpi and 48 hpi. (B) Venn diagrams showing overlaps of up-regulated genes in intestine samples collected at 24 hpi and 48 hpi. (C) KEGG enrichment analysis of the common down-regulated genes in intestine samples at 24 hpi and 48 hpi. Those significantly enriched pathways, which were involved in immune system, were highlighted in red. (D) KEGG enrichment analysis of the common up-regulated genes in intestine samples at 24 hpi and 48 hpi. The significantly enriched pathway "Complement and coagulation cascades" was highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).







(caption on next page)

Fig. 5. Comparative transcriptome analysis of the common DEGs in the skins. (A) Venn diagrams showing overlaps of down-regulated genes in skin samples collected at 24 hpi and 48 hpi. (B) Venn diagrams showing overlaps of up-regulated genes in skin samples collected at 24 hpi and 48 hpi. (C) KEGG enrichment analysis of the common down-regulated genes in skin samples at 24 hpi and 48 hpi. Those significantly enriched pathways, which were involved in immune system, were highlighted in red. (D) KEGG enrichment analysis of the common up-regulated genes in skin samples at 24 hpi and 48 hpi. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Comparative transcriptome analysis were further performed for DEGs at different time points after *S. agalactiae* XQ-1 infection. In the intestines, 1618 DEGs were downregulated both at 24 hpi and 48 hpi (Fig. 4A), whereas 1622 DEGs were upregulated both at 24 hpi and 48 hpi (Fig. 4B). In total 32 significantly enriched KEGG pathways were revealed for these 1618 common downregulated genes, which include 11 pathways involved in immune system and 14 pathways involved in metabolism (Supplementary data 1, Fig. 4C). These pathways involved in immune system include Th17 cell differentiation, Antigen processing and presentation, Cytokine-cytokine receptor interaction, NF-kappa B signaling pathway, T cell receptor signaling pathway, Hematopoietic cell lineage, Th1 and Th2 cell differentiation, Intestinal immune network for IgA production, Natural killer cell mediated cytotoxicity, Fc epsilon RI signaling pathway and PI3K-Akt signaling pathway (Fig. 4C). For 1622 common upregulated genes, 14 KEGG pathways were significantly enriched, which only a pathway "Complement and coagulation cascades" was involved in immune system category (Supplementary data 2, Fig. 4D).

In the skins, 672 and 428 DEGs both at 24 hpi and 48 hpi were downregulated and upregulated, respectively (Fig. 5A and B). In total 12 significantly enriched KEGG pathways were revealed for these 672 downregulated DEGs, which include 4 pathways involved in immune system, 3 pathways involved in digestive system and and 3 pathways involved in metabolism (Fig. 5C, Supplementary data 3). These pathways involved in immune system include Complement and coagulation cascades, Antigen processing and presentation, Intestinal immune network for IgA production and Hematopoietic cell lineage (Fig. 5C). For 428 upregulated DEGs, only 1 pathway were significantly enriched (Fig. 5D, Supplementary data 4).

3.6. Common pathways and unique genes involved in immune response are modulated in the intestines and skins

In our results, 3 pathways related to immune processes and immune-related components were significantly enriched for downregulated DEGs both in the intestines and skins, which include Antigen processing and presentation, Intestinal immune network for IgA production and Hematopoietic cell lineage (Figs. 4C and 5C). Comparative transcriptome analysis showed that a total of 20 genes involved in "Antigen processing and presentation" pathway were downregulated by *S. agalactiae* XQ-1 infection in the intestines (Fig. 6A) and 10 genes downregulated by *S. agalactiae* XQ-1 infection in the skins (Fig. 6B). For DEGs involved in "Hematopoietic cell lineage" pathway, 21 genes were downregulated by *S. agalactiae* XQ-1 infection in the intestines (Fig. 6C) and 9 genes downregulated in the skins (Fig. 6D). For DEGs involved in "Intestinal immune network for IgA production" pathway, 15 genes were downregulated by *S. agalactiae* XQ-1 infection in the intestines (Fig. 6E) and 7 genes downregulated in the skins (Fig. 6D). Interestingly, 11 genes were common to all groups, and these DEGs include 6 MHC2 genes, CIITA, ABCB3, CD74, CSF1R and PIGR (Fig. 6). We further determined that these 6 MHC2 genes were from the same transcript or different transcripts. The obvious differences in amino acids sequences suggested that these 6 MHC2 genes were different MHC2 genes. Among them, 3 MHC2 genes are novel proteins similar to MHC class II alpha chain, 2 MHC2 genes containing MHC_II_beta domain, 1 MHC2 gene containing IgC_MHC_I_alpha3 domain (Fig. 7).

3.7. Interaction network analysis of DEGs

To determine the interrelationship among these common DEGs at 24 hpi and 48 hpi, PPI analysis was performed based on STRING database. In the skins, 4 sub-networks were made by 19 genes, such as MMP9-JUNB-JUNBL-CEBPB for TNF signaling pathway, P38-DUSP1-DUSP2-DUSP4-DUSP5-DUSP6-JUND for MAPK signaling pathway, VEGFR1-VEGFR2-CDH5 for focal adhesion and F5-F10-VWF-SERPINE1 for complement and coagulation cascades (Fig. 8A). In the intestines, 10 sub-networks were made by 100 genes, which include Fanconi anemia pathway (26 genes), Base excision repair (14 genes), Mismatch repair (7 genes), Ubiquitin mediated proteolysis (4 genes), p53 signaling pathway (9 genes), AMPK signaling pathway (8 genes), Phagosome (7 genes), Spliceosome (7 genes), Leukocyte transendothelial migration (4 genes) and Complement and coagulation cascades (14 genes). The main DEGs in the sub-network of complement and coagulation cascades were C2, C3, C3a, C3b, C3–H1, F5, FGB, FGA, FGG, F13A1, SERPINE1, SERPINF2, PROC and PLAT (Fig. 8B).

3.8. Confirmation of differently expressed genes by qRT-PCR

We confirmed expression of 20 critical DEGs involved in complement and coagulation cascades by qRT-PCR in the skin samples. At 24 hpi, the expression of C3a, C5, C8a, C8b, C9, MBL, FGA, FGB, FGG, CFH, PLG, F13A1, VTN, f7i and A2M was in agreement with their transcript abundance changes determined by RNA-seq (Fig. 9A and Supplementary Table 2). At 48 hpi, the expression of all tested 20 genes was in agreement with transcriptome data, with 19 genes downregulated and 1 genes upregulated (Fig. 9B and Supplementary Table 2).

4. Discussion

In the present study, we report the characterization of transcriptome analysis for the intestines and skins from *S. agalactiae* XQ-1-infected and non-infected adult zebrafish. Analysis of intestine and skin transcriptome at 24 and 48 hpi after *S. agalactiae* XQ-1 infection, the number of DEGs in the intestines sharply increased at 24–48 hpi, while the DEG number in the skins was relative stable at 24–48 hpi. The possible reason could be that *S. agalactiae* XQ-1 was injected to visceral cavity by the intraperitoneal route, which led to dramatic bacterial proliferation in internal organs. However, only approximately 1/5–1/6 DEGs were shared between 24 hpi and 48 hpi in the intestines and skins, which suggested that different immune reactions were carried out.

4.1. Intestine transcriptomic analysis in response to S. agalactiae XQ-1 infection

During immune responses in response to pathogen infection, the intestinal tissues have not received as much attention as immune tissues such as spleen and head kidney. However within the intestines, the gut associated lymphoid tissue (GALT) contained numerous immune cell types such as macrophages, granulocytes, lymphocytes and plasma cells [36–38]. The intestine is also an important entry point for pathogens [38]. The intestinal immunity of fish is now subjected to many research, and several studies have been carried out using the whole transcriptome approach or microarray to examine intestinal responses to pathogens [39]. In the gilthead sea bream (*Sparus aurata L.*) response to

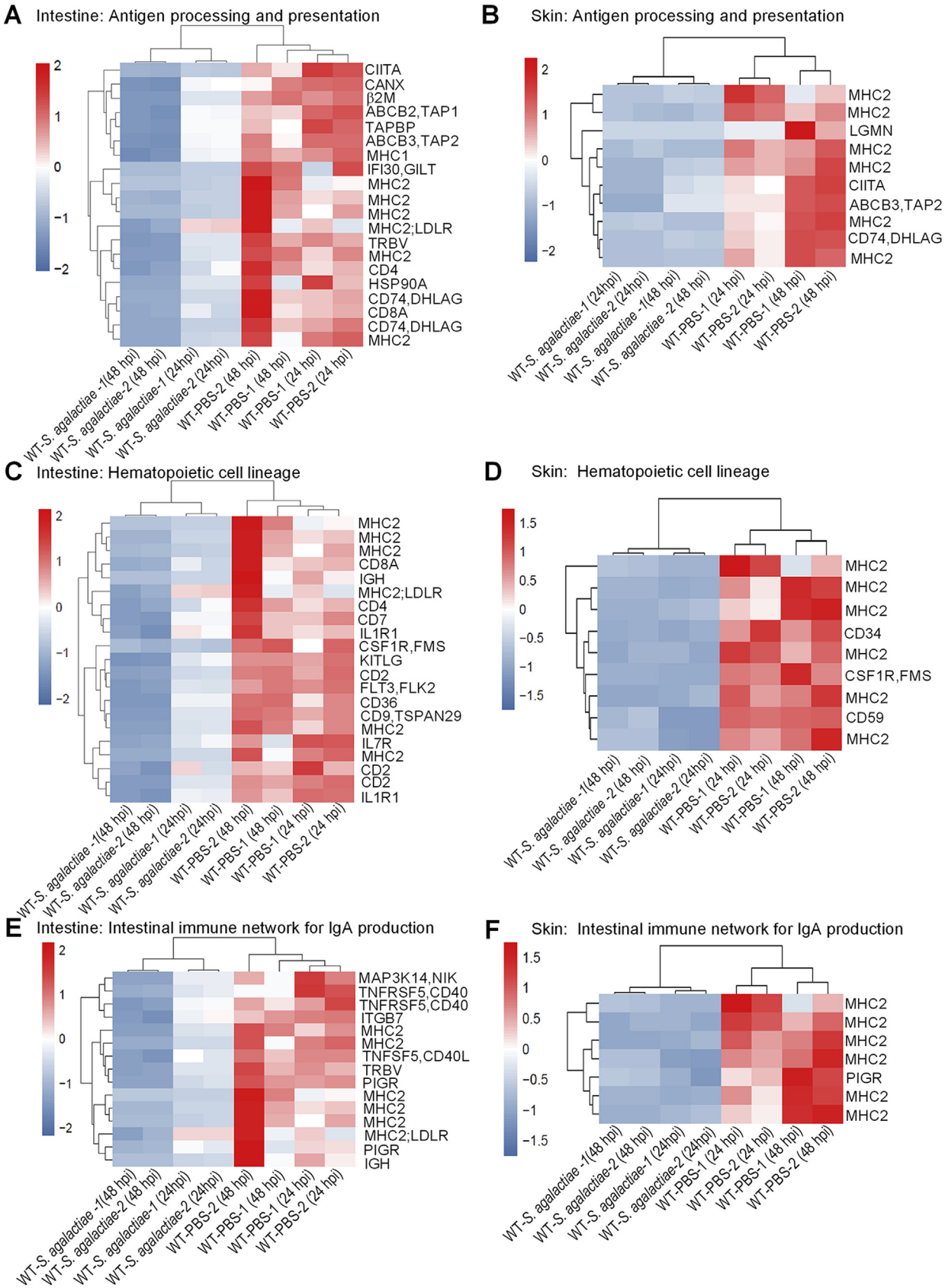


Fig. 6. Cluster analysis of DEGs. (A) The gene cluster for "Antigen processing and presentation" in intestine samples at 24 hpi and 48 hpi. (B) The gene cluster for "Antigen processing and presentation" in skin samples at 24 hpi and 48 hpi. (C) The gene cluster for "Hematopoietic cell lineage" in intestine samples at 24 hpi and 48 hpi. (D) The gene cluster for "Hematopoietic cell lineage" in skin samples at 24 hpi and 48 hpi. (E) The gene cluster for "Intestinal immune network for IgA production" in intestine samples at 24 hpi and 48 hpi. (F) The gene cluster for "Intestinal immune network for IgA production" in skin samples at 24 hpi and 48 hpi. At the left of this panel, a color key denotes the gradient scale of gene expression from low (blue) to high (red) degrees. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

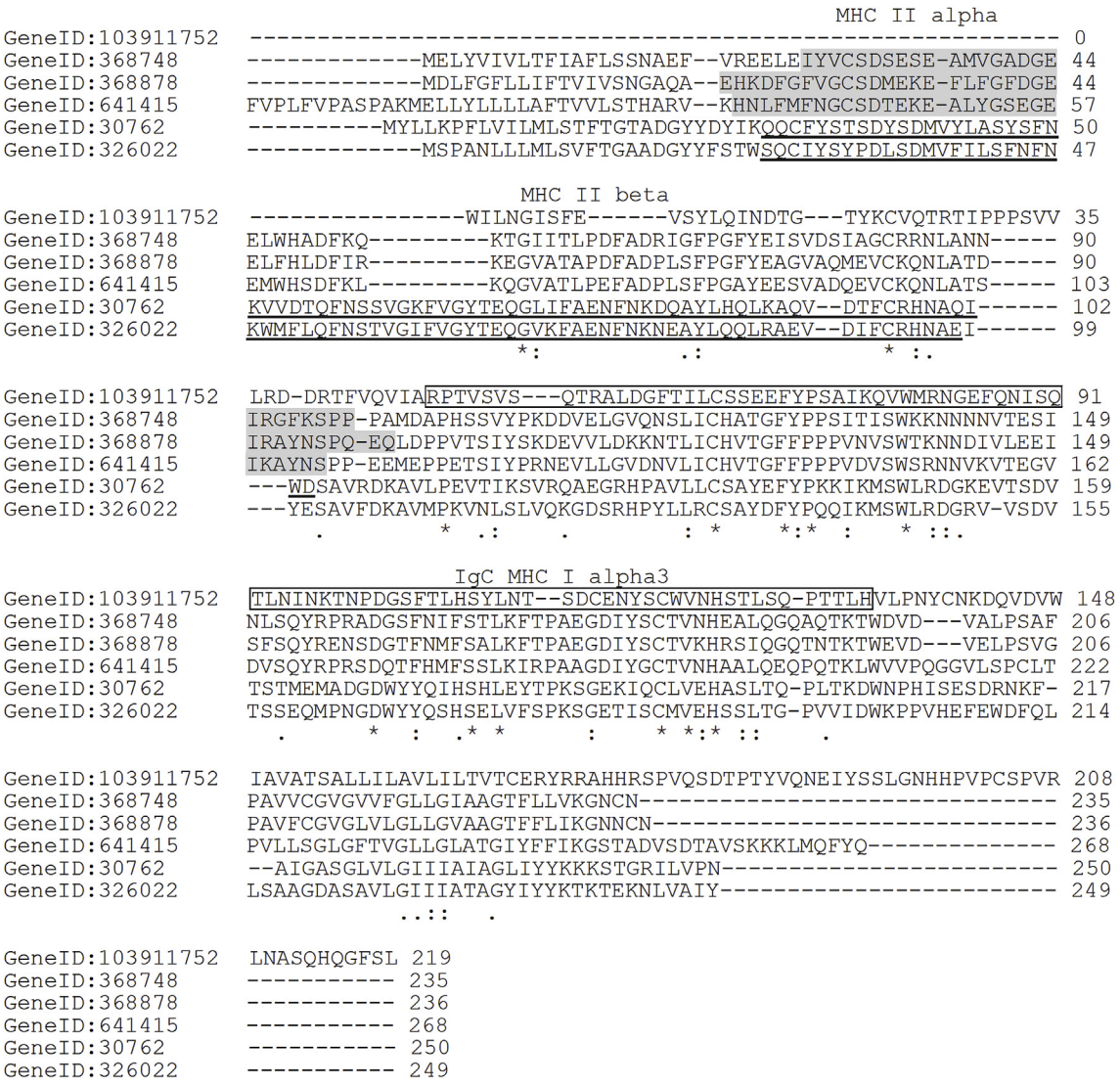


Fig. 7. The sequence alignments of 6 down-regulated MHC2 genes in all samples. The MHC II alpha domain was highlighted in grey, MHC II beta domain underlined, and IgC MHC I alpha3 domain in boxed.

chronic exposure to the myxosporean parasite *Enteromyxum leei*, many genes related to complement activation, acute phase response and cell adhesion (e.g. mannose binding lectin 2, complement C3-1, complement C1s, fibronectin, adhesive plaque matrix protein) were down-regulated in infected fish [40]. In the channel catfish following *Edwardsiella ictaluri* infection, nattectin, CD209 (DC-SIGN), MMP13, MMP9, tumor suppressor protein p53 and some innate immune factors including C1q-like genes, neurotoxin/CD59-like, liver-expressed antimicrobial peptide 2 (LEAP-2) and serum amyloid P were observed to be down-regulated [41]. In the turbot (*Scophthalmus maximus*) following *Vibrio anguillarum* challenge, a number of genes with putative roles in host intracellular pathogen recognition and innate immune genes involved in immune activation/inflammation were significantly down-regulated [42]. These results suggested a suppression of immune responses in the intestines.

In the present study, a number of genes involved in Toll-like receptor signaling pathway (45 genes), RIG-I-like receptor signaling pathway (33 DEGs), NOD-like receptor signaling pathway (65 DEGs), T cell receptor signaling pathway (50 DEGs), B cell receptor signaling pathway (40 DEGs), Antigen processing and presentation (39 DEGs), NF-kappa B signaling pathway (48 DEGs) and PI3K-Akt signaling pathway (125 DEGs) were significantly downregulated at 48 hpi (Supplementary Fig. 1-Fig. 8). This indicated that the intestine immune system of zebrafish underwent pathogen attack, which was confirmed by increasing mortality rate from 1 dpi to 4 dpi. Similarly, many genes involved in Necroptosis (73 DEGs), Ferroptosis (23 DEGs) and Apoptosis (71 DEGs) were also down-regulated at 48 hpi following *S. agalactiae* XQ-1 infection. In contrast, the overall depression for overwhelming majority of immune-ralated genes was paralleled by a clear increase in expression of those genes involved in Complement and coagulation cascades in the intestines (63 DEGs at 24 hpi and 28 DEGs at 48 hpi). The complement system is activated via three pathways (the lectin, classical or alternative pathway) and results in the opsonization of pathogens and activation and recruitment of immune cells to the site of injury [43]. Coagulation cascade can be activated through the extrinsic pathway or the intrinsic (contact activation) pathway [44]. It is obvious that all the pathways involved in complement system and coagulation cascade were activated in the intestines at 24 hpi (Supplementary Fig. 9), however only alternative and extrinsic pathways were activated in the intestines at 48 hpi. The higher bacteria numbers and lower DEGs involved in Complement and coagulation cascades at 48 hpi suggested that *S. agalactiae* XQ-1 also obstructed the activation of Complement and coagulation cascades to some extent.

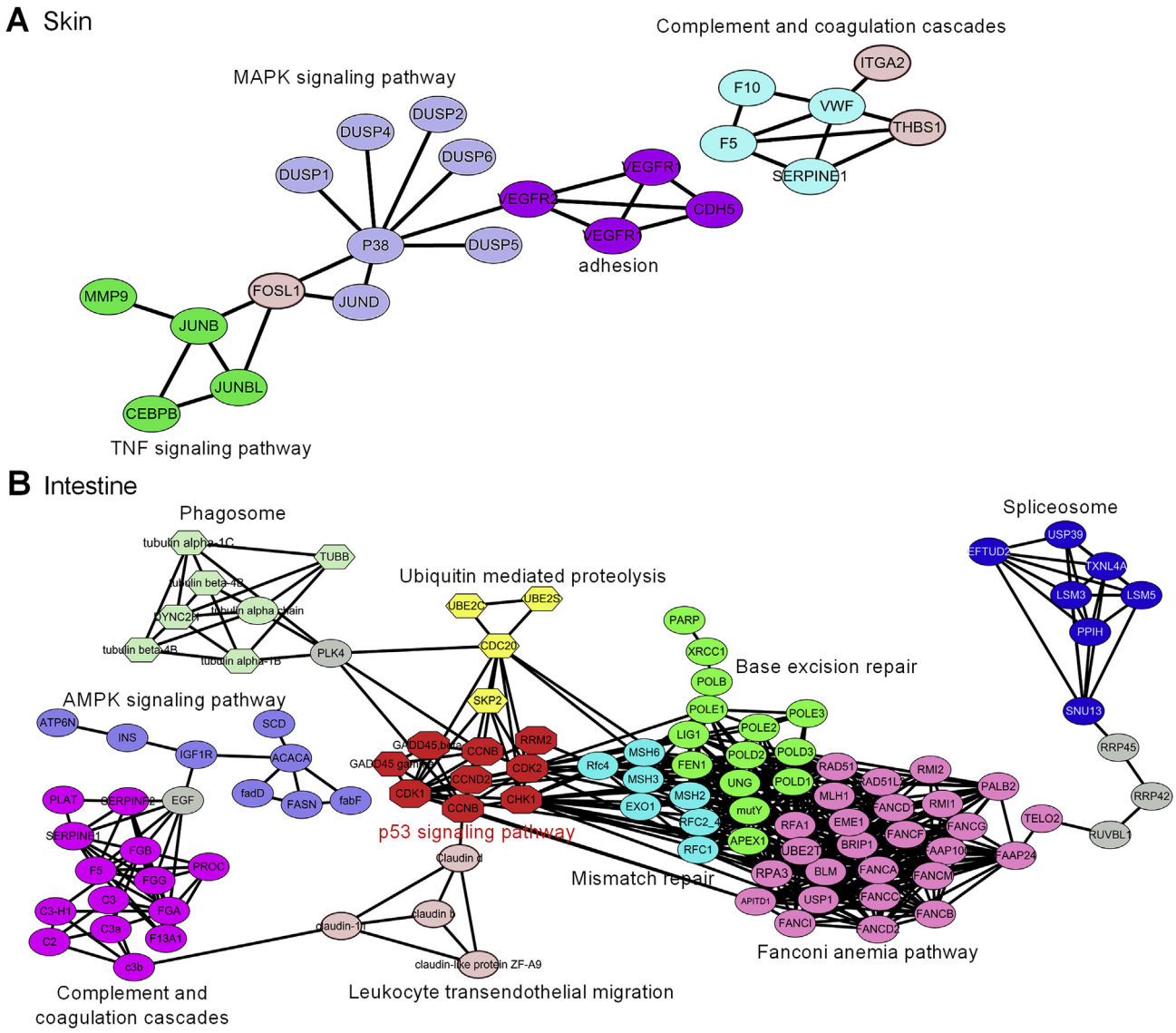


Fig. 8. Gene network functional interactions for the common DEGs at 24 hpi and 48hpi in the skins and the intestines. Cytoscape software was applied to visualize the protein network.

4.2. Skin transcriptomic analysis in response to S. agalactiae XQ-1 infection

Fish skin is the largest immunologically active mucosal organ, which provides first-line defense against environmental pathogens. In the face of ectoparasite infection, most genes involved in immune-related signaling pathways were induced in infected skin, which included Complement activation, Chemokines and chemokine receptors, Toll-like receptor signaling pathway, Antigen processing and presentation, T/B cell activation and proliferation pathways [45]. Similarly, transcriptomic analysis revealed that complement factors, pro-inflammatory cytokines, and antimicrobial genes were strikingly induced in the skin of infected fish in response to *Ichthyophthirius multifillis* infection [46]. In the present study, many genes involved in Intestinal immune network for IgA production (17 DEGs), Hematopoietic cell lineage (26 DEGs), Complement and coagulation cascades (38 DEGs) and Antigen processing and presentation (23 DEGs) were down-regulated at 48 hpi. This is consistent with the report from Yin et al., which showed that the immunological response was suppressed when fishes were in fatal conditions [47].

Interestingly, the downregulation of C1q, C3, C5, C6, C7, C8a, C8b, C8g, C9, MBL, FH, FI, FB, FD, MASP1/2 and CD59 in skin suggested that the lectin, classical and alternative pathways were inhibited, which were completely opposite of that in the intestines. Furthemore, more immune-related signaling pathways including Leukocyte transendothelial migration (42 DEGs), Cytokine-cytokine receptor interaction (49 DEGs), PI3K-Akt signaling pathway (69 DEGs), IL-17 signaling pathway (22 DEGs), MAPK signaling pathway (51 DEGs), TNF signaling pathway (25 DEGs), Complement and coagulation cascades (22 DEGs), Hematopoietic cell lineage (21 DEGs) and Jak-STAT signaling pathway (26 DEGs) were differently enriched for upregulated DEGs at 48 hpi (Table 5), which were also completely different from that in the intestines (Table 3). The different immune responses between the skins and the intestines may be due to the differences from external environment and internal environment, and the outcome of interaction between host and *S. agalactiae* XQ-1 at different organs.

5. Conclusions

This study characterized transcriptomic responses in the fish intestines and skins, two organs containing mucosal-associated lymphoid tissues. A number of genes involved in 23 immune-related signaling pathways were dysregulated during *S. agalactiae* XQ-1 infection. The

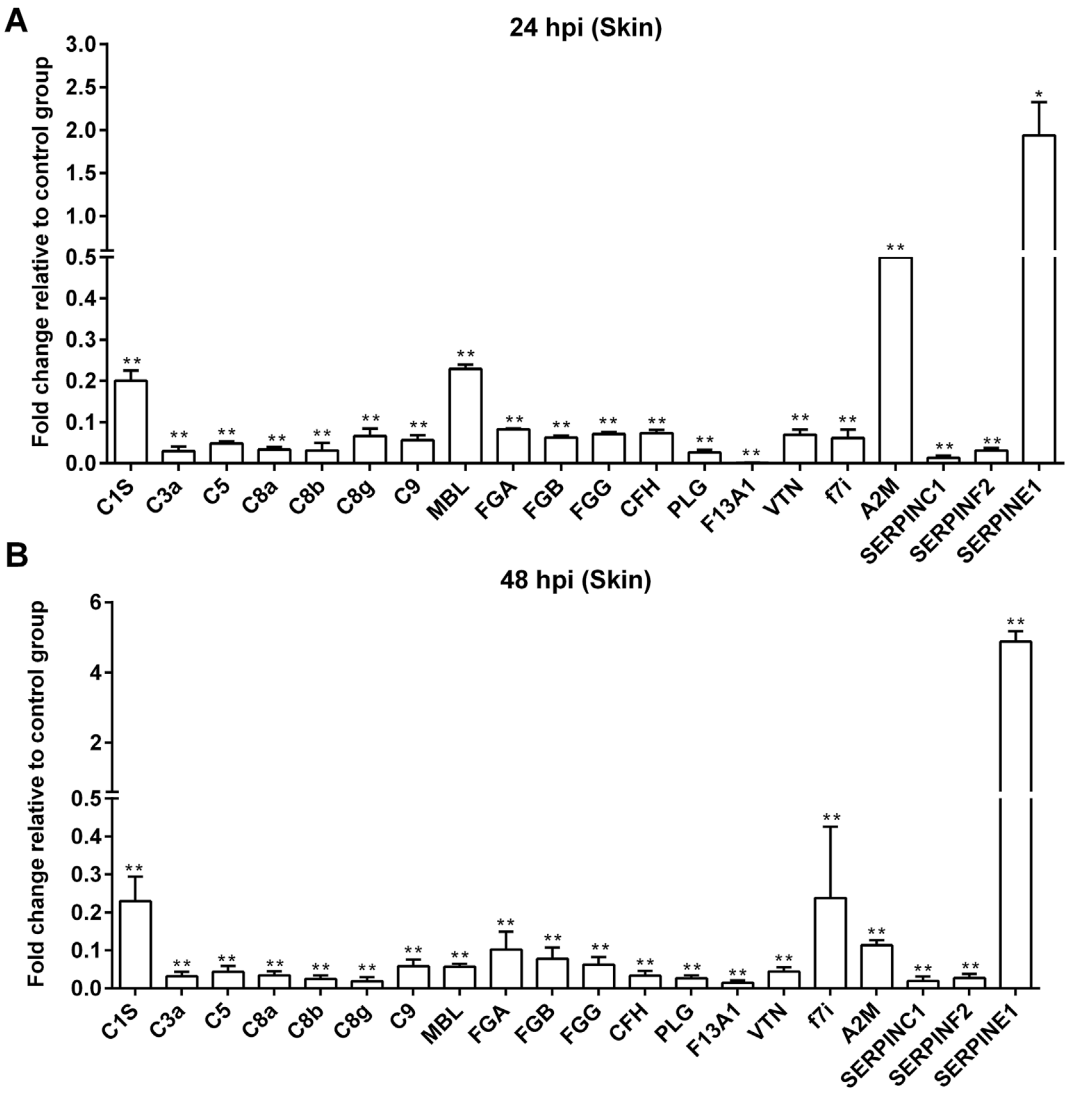


Fig. 9. Validation of transcriptome data by qRT-PCR in the skins at 24 hpi (A) and 48 hpi (B). Samples used in qRT-PCR experiment were previously used for the transcriptome experiment.

lower expression levels of most genes involved in immune-related signaling pathways in infected fish suggested that *S. agalactiae* XQ-1 suppressed the innate and adaptive immune responses of host in fatal conditions. The expression changes in Complement and coagulation cascades might be resulted from the outcome of interaction between host and *S. agalactiae* XQ-1. These data of WT zebrafish-*S. agalactiae* infection described in this study will be used for molecular exploration of the exact mechanisms of susceptible and resistant zebrafish in response to *S. agalactiae* XQ-1 infection.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fsi.2019.09.040.

References

[1] A. Saralahti, M. Rämet, Zebrafish and streptococcal infections, Scand. J. Immunol. 82 (3) (2015 Sep) 174–183.
[2] M.J. Walker, T.C. Barnett, J.D. McArthur, J.N. Cole, C.M. Gillen, A. Henningham, K.S. Sriprakash, M.L. Sanderson-Smith, V. Nizet, Disease manifestations and pathogenic mechanisms of Group A Streptococcus, Clin. Microbiol. Rev. 27 (2) (2014 Apr) 264–301.
[3] C.D. Acosta, J.J. Kurinczuk, D.N. Lucas, D.J. Tuffnell, S. Sellers, M. Knight, United Kingdom Obstetric Surveillance System. Severe maternal sepsis in the UK, 2011-2012: a national case-control study, PLoS Med. 11 (7) (2014 Jul 8) e1001672.
[4] D. van de Beek, J. de Gans, L. Spanjaard, M. Weisfelt, J.B. Reitsma, M. Vermeulen, Clinical features and prognostic factors in adults with bacterial meningitis, N. Engl. J. Med. 351 (18) (2004 Oct 28) 1849–1859.
[5] W.S. Lim, S.V. Baudouin, R.C. George, A.T. Hill, C. Jamieson, I. Le Jeune, Macfarlane JT., R.C. Read, H.J. Roberts, M.L. Levy, M. Wani, M.A. Woodhead, BTS guidelines for the management of community acquired pneumonia in adults: update 2009, Thorax 64 (Suppl 3) (2009 Oct) iii1-55s.
[6] T. Parks, L. Barrett, N. Jones, Invasive streptococcal disease: a review for clinicians, Br. Med. Bull. 115 (1) (2015 Sep) 77–89.
[7] K. Le Doare, P.T. Heath, An overview of global GBS epidemiology, Vaccine 31 (Suppl 4) (2013 Aug 28) D7–D12.
[8] J. Delamare-Debouteville, M. Kawasaki, E. Zoccola, C.M. Heath, R.O. Bowater, A.C. Barnes, Interactions of head-kidney leucocytes from giant grouper, Epinephelus lanceolatus, with pathogenic *Streptococcus agalactiae* strains from marine and terrestrial origins, Fish Shellfish Immunol. 90 (2019 Jul) 250–263.
[9] M.N.A. Amal, A. Ismail, M.Z. Saad, I.S. Md Yasin, N.S. Nasruddin, S.S. Mastor, M.H. Abdul Rahman, N. Mohamad, Study on *Streptococcus agalactiae* infection in Javanese medaka (Oryzias javanicus Bleeker, 1854) model, Microb. Pathog. 131

(2019 Jun) 47–52.
[10] A. Jaglarz, A. Gurgul, W.J. Leigh, J.Z. Costa, K.D. Thompson, Complete genome sequences of three fish-associated Streptococcus agalactiae isolates, Genome Announc. 6 (6) (2018 Feb 8).
[11] M.D. Baldissera, C.F. Souza, B.S. Parmeggiani, R.C.V. Santos, G. Leipnitz, K.L.S. Moreira, M.I.U.M. da Rocha, M.L. da Veiga, B. Baldisserotto, *Streptococcus agalactiae* impairs cerebral bioenergetics in experimentally infected silver catfish, Microb. Pathog. 111 (2017 Oct) 28–32.
[12] J.J. Evans, J.F. Bohnsack, P.H. Klesius, A.A. Whiting, J.C. Garcia, C.A. Shoemaker, S. Takahashi, Phylogenetic relationships among *Streptococcus agalactiae* isolated from piscine, dolphin, bovine and human sources: a dolphin and piscine lineage associated with a fish epidemic in Kuwait is also associated with human neonatal infections in Japan, J. Med. Microbiol. 57 (Pt 11) (2008 Nov) 1369–1376.
[13] X. Ye, J. Li, M.X. Lu, G.C. Deng, X.Y. Jiang, Y.Y. Tian, Y.C. Quan, Q. Jian, Identification and molecular typing of *Streptococcus agalactiae* isolated from pond-cultured tilapia in China, Fish. Sci. 77 (2011) 623–632.
[14] R.O. Bowater, J. Forbes-Faulkner, I.G. Anderson, K. Condon, B. Robinson, F. Kong, G.L. Gilbert, A. Reynolds, S. Hyland, G. McPherson, J.O. Brien, D. Blyde, Natural outbreak of Streptococcus agalactiae (GBS) infection in wild giant Queensland grouper, *Epinephelus lanceolatus* (Bloch), and other wild fish in northern Queensland, Australia, J. Fish Dis. 35 (3) (2012 Mar) 173–186.
[15] L.Y. Huang, K.Y. Wang, D. Xiao, D.F. Chen, Y. Geng, J. Wang, Y. He, E.L. Wang, J.L. Huang, G.Y. Xiao, Safety and immunogenicity of an oral DNA vaccine encoding Sip of *Streptococcus agalactiae* from Nile tilapia *Oreochromis niloticus* delivered by live attenuated Salmonella typhimurium, Fish Shellfish Immunol. 38 (1) (2014 May) 34–41.
[16] O. Noraini, M.Y. Sabri, A. Siti-Zahrah, Efficacy of spray administration of formalin-killed *Streptococcus agalactiae* in hybrid Red Tilapia, J. Aquat. Anim. Health 25 (2) (2013 Jun) 142–148.
[17] J.W. Pridgeon, P.H. Klesius, Development of live attenuated *Streptococcus agalactiae* as potential vaccines by selecting for resistance to sparfloxacin, Vaccine 31 (24) (2013 May 31) 2705–2712.
[18] G. Liu, J. Zhu, K. Chen, T. Gao, H. Yao, Y. Liu, W. Zhang, C. Lu, Development of *Streptococcus agalactiae* vaccines for tilapia, Dis. Aquat. Org. 122 (2) (2016 Dec 21) 163–170.
[19] A.A. Laith, M.A. Abdullah, W.W.I. Nurhafizah, H.A. Hussein, J. Aya, A.W.M. Effendy, M. Najiah, Efficacy of live attenuated vaccine derived from the *Streptococcus agalactiae* on the immune responses of Oreochromis niloticus, Fish Shellfish Immunol. 90 (2019 Jul) 235–243.
[20] H.C. Maisey, M. Hensler, V. Nizet, K.S. Doran, Group B streptococcal pilus proteins contribute to adherence to and invasion of brain microvascular endothelial cells, J. Bacteriol. 189 (4) (2007 Feb) 1464–1467.
[21] A. Tazi, O. Disson, S. Bellais, A. Bouaboud, N. Dmytruk, S. Dramsi, M.Y. Mistou, H. Khun, C. Mechler, I. Tardieux, P. Trieu-Cuot, M. Lecuit, C. Poyart, The surface protein HvgA mediates group B streptococcus hypervirulence and meningeal tropism in neonates, J. Exp. Med. 207 (11) (2010 Oct 25) 2313–2322.
[22] R. Mu, B.J. Kim, C. Paco, Y. Del Rosario, H.S. Courtney, K.S. Doran, Identification of a group B streptococcal fibronectin binding protein, SfbA, that contributes to invasion of brain endothelium and development of meningitis, Infect. Immun. 82 (2014) 2276–2286.
[23] C.F. Ken, C.N. Chen, C.H. Ting, C.Y. Pan, J.Y. Chen, Transcriptome analysis of hybrid tilapia (Oreochromis spp.) with *Streptococcus agalactiae* infection identifies Toll-like receptor pathway-mediated induction of NADPH oxidase complex and piscidins as primary immune-related responses, Fish Shellfish Immunol. 70 (2017 Nov) 106–120.
[24] J. Zhu, Q. Fu, Q. Ao, Y. Tan, Y. Luo, H. Jiang, C. Li, X. Gan, Transcriptomic profiling analysis of tilapia (*Oreochromis niloticus*) following *Streptococcus agalactiae* challenge, Fish Shellfish Immunol. 62 (2017 Mar) 202–212.
[25] J. Zhu, X. Gan, Q. Ao, X. Shen, Y. Tan, M. Chen, Y. Luo, H. Wang, H. Jiang, C. Li, Basal polarization of the immune responses to *Streptococcus agalactiae* susceptible and resistant tilapia (*Oreochromis niloticus*), Fish Shellfish Immunol. 75 (2018 Apr) 336–345.
[26] H.R. Park, S.M. Harris, E. Boldenow, R.C. McEachin, M. Sartor, M. Chames, R. Loch-Caruso, Group B streptococcus activates transcriptomic pathways related to premature birth in human extraplacental membranes in vitro, Biol. Reprod. 98 (3) (2018 Mar 1) 396–407.
[27] A.P. Sbardella, M.M.D.C.A. Weller, I. Fonseca, N.B. Stafuzza, P.A. Bernardes, F.F. E Silva, M.V.G.B. da Silva, M.F. Martins, D.P. Munari, RNA sequencing differential gene expression analysis of isolated perfused bovine udders experimentally inoculated with *Streptococcus agalactiae*, J. Dairy Sci. 102 (2) (2019 Feb) 1761–1767.
[28] L.I. Zon, Zebrafish: a new model for human disease, Genome Res. 9 (2) (1999 Feb) 99–100.
[29] A.M. van der Sar, B.J. Appelmelk, C.M. Vandenbroucke-Grauls, W. Bitter, A star with stripes: zebrafish as an infection model, Trends Microbiol. 12 (10) (2004 Oct) 451–457.
[30] D.G. Howe, Y.M. Bradford, A. Eagle, D. Fashena, K. Frazer, P. Kalita, P. Mani, R. Martin, S.T. Moxon, H. Paddock, C. Pich, S. Ramachandran, L. Ruzicka, K. Schaper, X. Shao, A. Singer, S. Toro, C. Van Slyke, M. Westerfield, The Zebrafish Model Organism Database: new support for human disease models, mutation details, gene expression phenotypes and searching, Nucleic Acids Res. 45 (D1) (2017 Jan 4) D758–D768.
[31] H. Patterson, A. Saralahti, M. Parikka, S. Dramsi, P. Trieu-Cuot, C. Poyart, S. Rounioja, M. Rämet, Adult zebrafish model of bacterial meningitis in *Streptococcus agalactiae* infection, Dev. Comp. Immunol. 38 (3) (2012 Nov) 447–455.
[32] A. Saralahti, H. Piippo, M. Parikka, B. Henriques-Normark, M. Rämet, S. Rounioja, Adult zebrafish model for pneumococcal pathogenesis, Dev. Comp. Immunol. 42 (2) (2014 Feb) 345–353.
[33] B.R. Hanson, D.L. Runft, C. Streeter, A. Kumar, T.W. Carion, M.N. Neely, Functional analysis of the CpsA protein of *Streptococcus agalactiae*, J. Bacteriol. 194 (7) (2012 Apr) 1668–1678.
[34] Z. Wang, C. Guo, Y. Xu, G. Liu, C. Lu, Y. Liu, Two novel functions of hyaluronidase from *Streptococcus agalactiae* are enhanced intracellular survival and inhibition of proinflammatory cytokine expression, Infect. Immun. 82 (6) (2014 Jun) 2615–2625.
[35] B. Lin, S. Chen, Z. Cao, Y. Lin, D. Mo, H. Zhang, J. Gu, M. Dong, Z. Liu, A. Xu, Acute phase response in zebrafish upon *Aeromonas salmonicida* and *Staphylococcus aureus* infection: striking similarities and obvious differences with mammals, Mol. Immunol. 44 (4) (2007 Jan) 295–301.
[36] J.H. Rombout, L. Abelli, S. Picchietti, G. Scapigliati, V. Kiron, Teleost intestinal immunology, Fish Shellfish Immunol. 31 (5) (2011 Nov) 616–626.
[37] I. Salinas, Y.A. Zhang, J.O. Sunyer, Mucosal immunoglobulins and B cells of teleost fish, Dev. Comp. Immunol. 35 (12) (2011 Dec) 1346–1365.
[38] I. Salinas, D. Parra, Fish mucosal immunity: intestine, Mucosal Heal Aquac. (2015) 135–170.
[39] S.A. Martin, C.E. Dehler, E. Król, Transcriptomic responses in the fish intestine, Dev. Comp. Immunol. 64 (2016 Nov) 103–117.
[40] G.C. Davey, J.A. Calduch-Giner, B. Houeix, A. Talbot, A. Sitjà-Bobadilla, P. Prunet, J. Pérez-Sánchez, M.T. Cairns, Molecular profiling of the gilthead sea bream (*Sparus aurata L.*) response to chronic exposure to the myxosporean parasite Enteromyxum leei, Mol. Immunol. 48 (15–16) (2011 Sep) 2102–2112.
[41] C. Li, Y. Zhang, R. Wang, J. Lu, S. Nandi, S. Mohanty, J. Terhune, Z. Liu, E. Peatman, RNA-seq analysis of mucosal immune responses reveals signatures of intestinal barrier disruption and pathogen entry following *Edwardsiella ictaluri* infection in channel catfish, Ictalurus punctatus, Fish Shellfish Immunol. 32 (5) (2012 May) 816–827.
[42] C. Gao, Q. Fu, B. Su, S. Zhou, F. Liu, L. Song, M. Zhang, Y. Ren, X. Dong, F. Tan, C. Li, Transcriptomic profiling revealed the signatures of intestinal barrier alteration and pathogen entry in turbot (*Scophthalmus maximus*) following Vibrio anguillarum challenge, Dev. Comp. Immunol. 65 (2016 Dec) 159–168.
[43] M. Matsushita, The complement system of agnathans, Front. Immunol. 9 (2018 Jun 18) 1405.
[44] R. Wiegner, S. Chakraborty, M. Huber-Lang, Complement-coagulation crosstalk on cellular and artificial surfaces, Immunobiology 221 (10) (2016 Oct) 1073–1079.
[45] Y. Hu, A. Li, Y. Xu, B. Jiang, G. Lu, X. Luo, Transcriptomic variation of locally-infected skin of Epinephelus coioides reveals the mucosal immune mechanism against *Cryptocaryon irritans*, Fish Shellfish Immunol. 66 (2017 Jul) 398–410.
[46] X. Zhang, L. Ding, Y. Yu, W. Kong, Y. Yin, Z. Huang, X. Zhang, Z. Xu, The change of teleost skin commensal microbiota is associated with skin mucosal transcriptomic responses during parasitic infection by *Ichthyophthirius multifillis*, Front. Immunol. 9 (2018 Dec 18) 2972.
[47] F. Yin, Q. Gao, B. Tang, P. Sun, K. Han, W. Huang, Transcriptome and analysis on the complement and coagulation cascades pathway of large yellow croaker (*Larimichthys crocea*) to ciliate ectoparasite *Cryptocaryon irritans* infection, Fish Shellfish Immunol. 50 (2016 Mar) 127–141.