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Dual RNA-Seq uncovers the function of an ABC transporter gene in the host-pathogen interaction between *Epinephelus coioides* and *Pseudomonas plecoglossicida*

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ABSTRACT

As an important pathogen in aquaculture, *Pseudomonas plecoglossicida* has caused heavy losses. The expression of an ABC transporter gene-*L321_23611* of *P. plecoglossicida* at 18 °C was found significant higher than those at 28 °C by RNA-seq and qRT-PCR. RNAi significantly reduced the content of *L321*_23611 mRNA in *P. plecoglossicida* with a maximal decrease of 89.2%. Compared with the wild type strain, the infection of *L321_23611*-RNAi strain resulted in the reduction in mortality and the onset time delay of a kind of marine teleosts, *Epinephelus coioides*. The results of dual RNA-seq showed that the RNAi of *L321_23611* resulted in a significant change in both pathogen and host transcriptome in the spleens of infected *E. coioides*. The result of GO and KEGG analysis from dual RNA-seq data showed both host genes of chemokine signaling pathway, coagulation and complement system, hematopoietic cell lineage pathway as well as hemoglobin complex GO term and pathogenic genes of bacterial-type flagellum-dependent cell mortality GO term and flagellar assembly, biosynthesis of amino acids and lysine biosynthesis systems pathways were mainly affected by *L321_23611* gene of *P. plecoglossicida*. The results indicated that: 1. ABC transporter gene-*L321_23611* was a virulent gene of *P. plecoglossicida*. 2. Both the activation of the host immune pathways and depression of pathogenic virulence-related pathways facilitated *E. coioides* to remove *L321_23611*-RNAi strain than the wild type strain of *P. plecoglossicida*.

1. Introduction

The infection process is a fierce battle between the pathogen and the host, in which both the pathogen and the host must do their best to win [1]. In order to win this life-and-death struggle, both the pathogen and the host must mobilize all available resources, and all changes will be reflected in their respective transcriptome profiles [2]. Therefore, synchronous detecting of the transcriptome profiles of the two interacting species during infection will obtain a comprehensive understanding of the host immune response and the pathogenic mechanisms [3]. Recently, dual RNA-Seq analyses were performed successfully in *Salmonella typhimurium* with HeLa cells [4], *Streptococcus pneumonia* with lung epithelial cells [5] and *Epinephelus coioides* with *Pseudomonas plecoglossicida* [6], and discoverd many new information about host immune response to pathogen infection from the perspective of host-pathogen interaction [4–6].

Epinephelus coioides is marine teleosts which widely distributed in Indo-West Pacific [7]. *E. coioides* is an economy important fish and widely culutured in Southern China. *E. coioides* farming often encounters epidemics and suffers serious economic losses [8]. *P. plecoglossicida* is known as the causative agent of the "Visceral White Spot Disease" of marine teleosts, such as large yellow croaker (*Pseudosciaena crocea*) [9] and *E. coioides* [10] in China and causing serious economic losses. The infective disease caused by *P. plecoglossicida* is temperature-dependent and most developed during the seawater temperature range from 15 to 20 °C [11]. In order to reveal the underlying pathogenicity, the transcriptome analysis were conducted from *P. plecoglossicida* cultured under 18 and 28 °C, respectively [10], and the result has been deposited in the NCBI Sequence Read Archive (accession number SRP107111). The results showed that the expression level of *L321_23611* gene of *P.plecoglossicida* was significantly higher under 18 °C (temperature of high incidence of disease) than that under 28 °C

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(temperature of rapid growth of *P. plecoglossicida*). Thus, *L321_23611* gene was supposed to play roles in the pathogenicity of *P. plecoglossicida* and host-pathogen interaction.

L321_23611 encodes one member of the ATP binding cassette (ABC) transporter superfamily, which is one of the largest classes of transporters [12] and responsible for the ATP-powered translocation of many substrates, ranging from ions to macromolecules, across membranes [13]. ABC transporters are found in all species from the lowliest microbe to man, and play a wide variety of physiological roles, and are of very considerable medical and economic importance [14]. In microorganisms, ABC transporters are central to antibiotic and antifungal resistance. This family are involved in plenty of physiological processes [15–19], and are directly connected to human diseases [20], tumor resistance to chemotherapy [21], and bacterial virulence [22]. Up to the present, many genes such as *glnPQ* [22], *mntA* [20], *htsA* [23] encoding ABC transporter have been demonstrated to be key virulence determinants in bacterial pathogen. However, to our present knowledge, the effect of *L321_23611* on the immune response of teleosts host as well as it roles in host-pathogen interaction is still unknown.

Considering the high mortality of cultured marine fish caused by *P. plecoglossicida* [9,10] and the potential role of *L321_23611* gene in the host-pathogen interaction, the aim of this study is to elucidate the immune response of *E. coioides* to *L321_23611* gene of *P. plecoglossicida* from the perspective of host-pathogen interaction. Bacterial invasion and colonization of *P. plecoglossicida* were mainly occurred at spleen [6], which also presented the evidently different symptoms between infected spleens and was regarded as the best organ for investigating the host-pathogen interaction of *E. coioides* and *P. plecoglossicida*. In the present study, the *L321_23611* gene was stable silenced by pCM130/tac vector, and the virulence of wild-type strain and *L321_23611*-RNAi strain of *P. plecoglossicida* to *E. coioides* were assayed, and the spleens of *E. coioides* infected by wild-type strain and *L321_23611*-RNAi strain of *P. plecoglossicida* were subjected to dual RNA-Seq, final, both transcriptome data of host and pathogen from the spleens of *E. coioides* infected by RNAi strain of *P. plecoglossicida* were compared with the counterpart infected by wild strain of *P. plecoglossicida*.

2. Material and methods

2.1. Bacterial strains and culture conditions

The pathogenic *P. plecoglossicida* strain (NZBD9) was isolated from a spleen of naturally infected *P. crocea.* The isolate was identified as the pathogen by infection experiment and was identified as *P. plecoglossicida* by time-of-flight mass spectrometry [11]. *P. plecoglossicida* was incubated in LB (Luria Bertani) broth overnight at 18 °C or 28 °C with shaking at 220 rpm. *Escherichia coli* DH5α (TransGen Biotech, Beijing, China) was incubated in LB broth (37 °C, 220 rpm).

2.2. Construction of P. plecoglossicida RNAi strain

Construction of RNAi strain was carried out according to the previous described methods [6,24,25]. Invitrogen Block-iT RNAi Designer (http://rnaidesigner.thermofisher.com/rnaiexpress/setOption.do?designOption=shrna&pid=7085871032206845) was used to design five shRNA sequences to silence *L321_2361* genes(Supplementary Table 1). The five short hairpin RNA (shRNA) sequences were produced by Shanghai Generay Biotech Co., Ltd (Shanghai, China). The annealed oligonucleotides were combined with pCM130/tac vectors. Competent *E. coli* DH5α cells were imported from the recombinant pCM130/tac vectors via heat-shock and then were extracted and transferred by electroporation into *P. plecoglossicida* [26]. Finally, qRT-PCR was used to reckon the individual expression level of *L321_23611* gene of each strains. The strain with optimal silencing efficiency was selected for following experiments.

2.3. Artificial infection and sampling

The fish experiments were approved by the Animal Ethics Committee of Jimei University (Acceptance No. JMULAC201159) and strictly consistent with the recommendations in the 'Guide for the Care and Use of Laboratory Animals' established by the National Institutes of Health. Healthy weight-matched *E. coioides*(obtained from Zhangzhou, China) and accommodated for one week under the experimental conditions at 18 ± 1 °C before infection [27]. Twenty fish were used in each infection group of *L321_23611*-RNAi strain or wild type strain of *P. plecoglossicida*. The survival test was performed by intrapleurally injecting *E. coioides* with 10^3 colony forming units per gram (cfu/g) of *P. plecoglossicida* (wild-type strain or the mutant strain). Morbidity and mortality of infected fish were observed routinely for 20 days. The dead fish were removed in time. Spleens of six fish infected with wild-type strain or mutant strain of *P. plecoglossicida* at 2 days post infection (dpi) were sampled for dual RNA-Seq. Two individual spleens were mixed as one sample. For the tissue distribution assays, Spleens of six *E. coioides* (three fish in each group) infected with wild-type strain or mutant strain of *P. plecoglossicida* were sampled at 24, 48, 72 and 96 hpi, respectively.

2.4. DNA and RNA extraction

Genomic DNA was extracted from infected spleens by using the EasyPure Marine Animal Genomic DNA Kit (TransGen Biotech, Beijing, China). 16s rDNA from the DNA samples were used for pathogen load assay by qRT-PCR. Total RNA was extracted by the TRIzol reagent (Invitrogen, USA) following the user manual for detection of genes expression. After removing the mix genomic DNA with the Turbo DNA-free DNase (Ambion, Austin, TX, USA), the RNA quality was assayed using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA), followed by removing the rRNA using a Ribo ZerorRNA Removal Kit (Epicentre, USA). Agarose gel electrophoresis was used to verify the quality of the remaining RNA. The cDNA was synthesized using the TaKaRa PrimeScriptTM RT-PCR Kit (TaKaRa Bio Group, Japan) and stored at −20 °C. The copy number of the 16s rDNA gene per milligram of tissue was used to estimate the pathogen load in spleens.

2.5. qRT-PCR

QRT-PCR was performed using a QuantStudio 6 Flex (Life Technologies). All primer sequences are presented in Supplementary Table 2. The expression of *P. plecoglossicida* genes was normalized against *gyrB* and the expression of mRNA in *E. coioides* was normalized against β-actin [27]. The $2^{-\Delta\Delta Ct}$ method was taken into calculation of the relative level of gene expression.

2.6. Transcriptome analysis

2.6.1. Library preparation and illumina sequencing

The dual RNA-seq library was prepared using the protocols powered by the TruSeqTM RNA sample preparation Kit (Illumina, USA). The cleansed RNA sample was fragmented using fragmentation buffer, and Superscript double-stranded cDNA synthesis kit (Invitrogen, Carlsbad, USA) was used to cDNA synthesis. End reparation, phosphorylation and poly (A) addition were strictly conducted before Phusion DNA polymerase (NEB) was used to amplify the cDNA library. Library quality was validated by an Agilent 2100 Bioanalyzer (Agilent Technologies). RNA-Seq was performed on the Illumina HiSeq4000 sequencing platforms at Majorbio Biotech Co., Ltd (Shanghai, China).

2.6.2. Processing and mapping of reads

Sickle(https://github.com/najoshi/sickle) and SeqPrep (https://github.com/jstjohn/SeqPrep) were used to trim and quality control of raw Illumina reads with the optimal settings. Clean data were mapped

against the reference genome reads of NZBD9 (NCBI RefSeq accession numbers: SRP062985) using Bowtie2 [28].

2.6.3. De novo assembly and annotation of mRNAs

Mapped reads were considered as reads of *P. plecoglossicida*, and remaining clean reads from the infected spleens were regarded as a *E. coioides* reads, which was assembled de novo into unigenes by Trinity [29]. All unigenes were previously aligned to the bacterial NCBI non-redundant (NR) protein database to entirely remove prokaryote contamination. Furthermore, processed reads of *P. plecoglossicida* and *E. coioides* unigenes were mapped against SWISS-PROT, STRING, NCBI NR protein and KEGG databases. Identification of the proteins, which shared the optimal sequence similarity with the identified unigenes was carried out by BLASTX. Blast2GO program (http://www.blast2go.com/b2ghome) was used for GO annotations [30] while KEGG program was used for kegg pathway annotations (http://www.genome.jp/kegg/) [31].

2.7. Analysis of differential gene expression

Analysis of expression of RNA-seq data from *E. coioides* were carried out on the basis of the reference transcriptome annotation described above (annotation of mRNA in *E. coioides*) and annotations from NCBI (NZ_ASJX00000000.1), respectively. After acquiring uniquely mapped read counts, the package edgeR [32] was used to test for differently expressed genes (DEGs) under the criteria of $|\log_2 \text{fold change}| \geq 1$ and a false discovery rate (FDR) < 0.05.

2.8. Statistical analyses

All of the data were expressed as the means ± standard deviation (SD) from at least three independent experiments. Data analysis was carried out by the SPSS 17.0 software (Chicago, USA) with one-way analysis of variance with Dunnett's test. P values < 0.05 was considered statistically significant.

2.9. Data access

The RNA sequencing reads data were deposited at the GenBank SRA database under the accession numbers PRJNA515591 and SRP115064.

3. Results

3.1. Construction of the L321_23611-RNAi strain

The qRT-PCR results showed that the expression level of *L321_23611* gene in *P. plecoglossicida* cultured under 18 °C was more than 2 times higher than that under 28 °C, which was consistent with the result of transcriptome analysis (Fig. 1A). All of the five shRNAs resulted in the reduction of the content of *L321*_23611 mRNA in *P. plecoglossicida* with different efficiency. The reduction of relative expression level of *L321_23611* gene by *L321*_23611-shRNA-91, *L321*_23611-shRNA-93, *L321*_23611-shRNA-389, *L321*_23611-shRNA-911 and *L321*_23611-shRNA-920 was 69.2%, 18%, 60.9%, 89.2% and 55.3%, respectively (Fig. 1B). *L321*_23611-shRNA-911 strain (named the *L321_23611*-RNAi strain below) exhibited the best efficiency for silencing the *L321_23611* gene and was picked up for following study.

3.2. The effect of the L321_23611 gene on the pathogenicity of P. plecoglossicida

Compared with wild-type strain, injection of *L321_23611*-RNAi strain of *P. plecoglossicida* could delay the onset of the disease of *E. coioides* by one day and decrease the mortality. The first death of *E. coioides* infected by wild-type strain of *P. plecoglossicida* was recorded at 3dpi, which was 1 day earlier than the first death of *E. coioides* injected with *L321_23611*-RNAi strain. All *E. coioides* infected by wild-type strain of *P. plecoglossicida* died at 6dpi, while the last death of *E. coioides* injected with *L321_23611*-RNAi strain was recorded at 7 dpi and 8% of survival rate was recorded up to 20 dpi (Fig. 2A). At 1dpi and 2dpi, the pathogen load of *L321_23611*-RNAi strain of *P. plecoglossicida* in the spleen were close to those of wild type strain, but at 3dpi and 4dpi, the pathogen load of *L321_23611*-RNAi strain of *P. plecoglossicida* in the spleen were much lower than those of wild type strain (Fig. 2B).

3.3. Tissue dual RNA-Seq of spleen of infected E. coioides

The tissue dual RNA-seq program systematically cataloged the transcriptome wild type strain and RNAi strain of *P. plecoglossicida* grown in the spleen of infected *E. coioides*. The base composition along the reads is proper, and N% is within the reasonable range. The error ratio of the reads along the position is less than 0.1% and regarded as highly acceptable. The data reproducibility of the three biological replicates in both infected spleens is satisfactory. For further analysis, all data are separated by mapping against bacterial reference genome into host and pathogenic data, respectively. The gene expression analysis was conducted by edgeR, and changed genes subjected to the criteria of FDR < 0.05 and $|\log_2 FC| > 1$ were regarded as significantly statistical change. Validation of dual RNA-seq was conducted by qRT-PCR.

3.4. Different expression genes (DEGs) in E. coioides

Of 183,843 profiled mRNAs of *L321_23611*-RNAi strain-infected spleens, 20,460 were significantly altered in abundance in contrast to

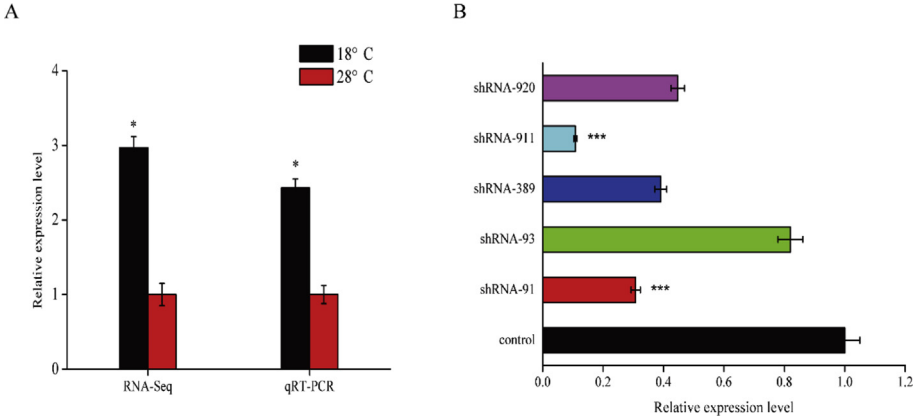

Fig. 1. Construction of the *L321_23611*-RNAi strain. A, the relative expression level of gene *L321_23611* under 18 and 28 °C. B, the gene-silence effectiveness of each shRNAs, respectively.

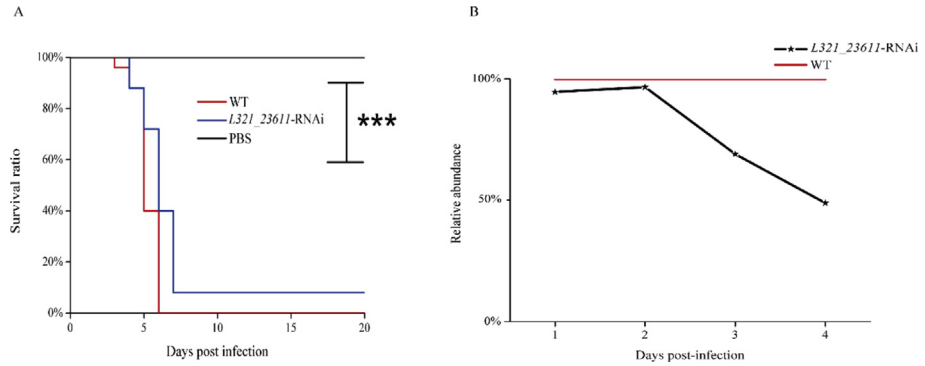

Fig. 2. The effect of the gene *L321_23611* during infection. A, mortality during infection with wild-type(wt), *L321_23611*-RNAi strain and PBS. B, the relative pathogen load between infected spleens.

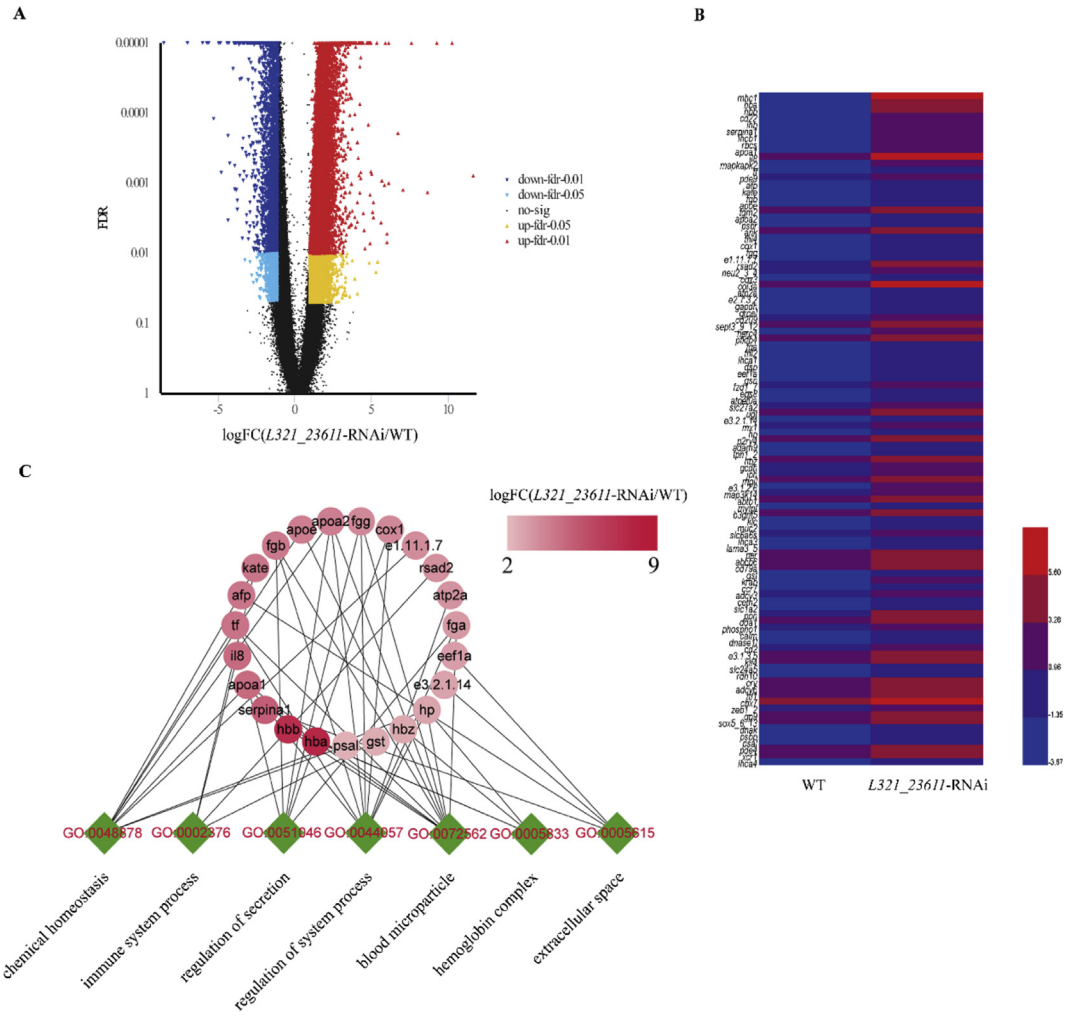

Fig. 3. Different Expression Genes (DEGs) in *E. coioides*. A, vocalno of DEGs between spleens infected by *L321_23611*-RNAi strain and wild type strain of *P. plecoglossicida*. B, heat map of top 100 DEGs. C, the relationship between 23 up-regulated DEGs and 7 GO terms.

wild-type-infected spleens. Of 20,460 DEGs identified, 868 DEGs were annotated by GO (Supplementary Figs. 1) and 2144 DEGs were annotated by KEGG (Supplementary Fig. 2). The different expression genes of *E. coioides* among spleens infected by *L321_23611*-RNAi strain and wild type strain of *P. plecoglossicida* are showed in Fig. 3A. In 20,460 DEGs, 14,928 were up-regulated 5532 were down-regulated. Based on the criteria mentioned above, the top 100 up-regulated DEGs were selected and presented in Figure 3B. Of the top 100 up-regulated DEGs, 77 failed to map against GO terms, and 23 successfully mapped against 7 GO terms, which are blood microparticle, chemical homeostasis, extracellular space, hemoglobin complex, immune system process, regulation of secretion and regulation of system process, respectively. Relations between genes and GO terms are presented in Figure 3C. Among the 7 GO terms, the hemoglobin complex is highly direct immune-related one.

In terms of KEGG database, 49 of the top 100 up-regulated DEGs were enriched in 18 KEGG pathways. Compared with the wild type strain, all of the 18 KEGG pathways were up-regulated in spleen

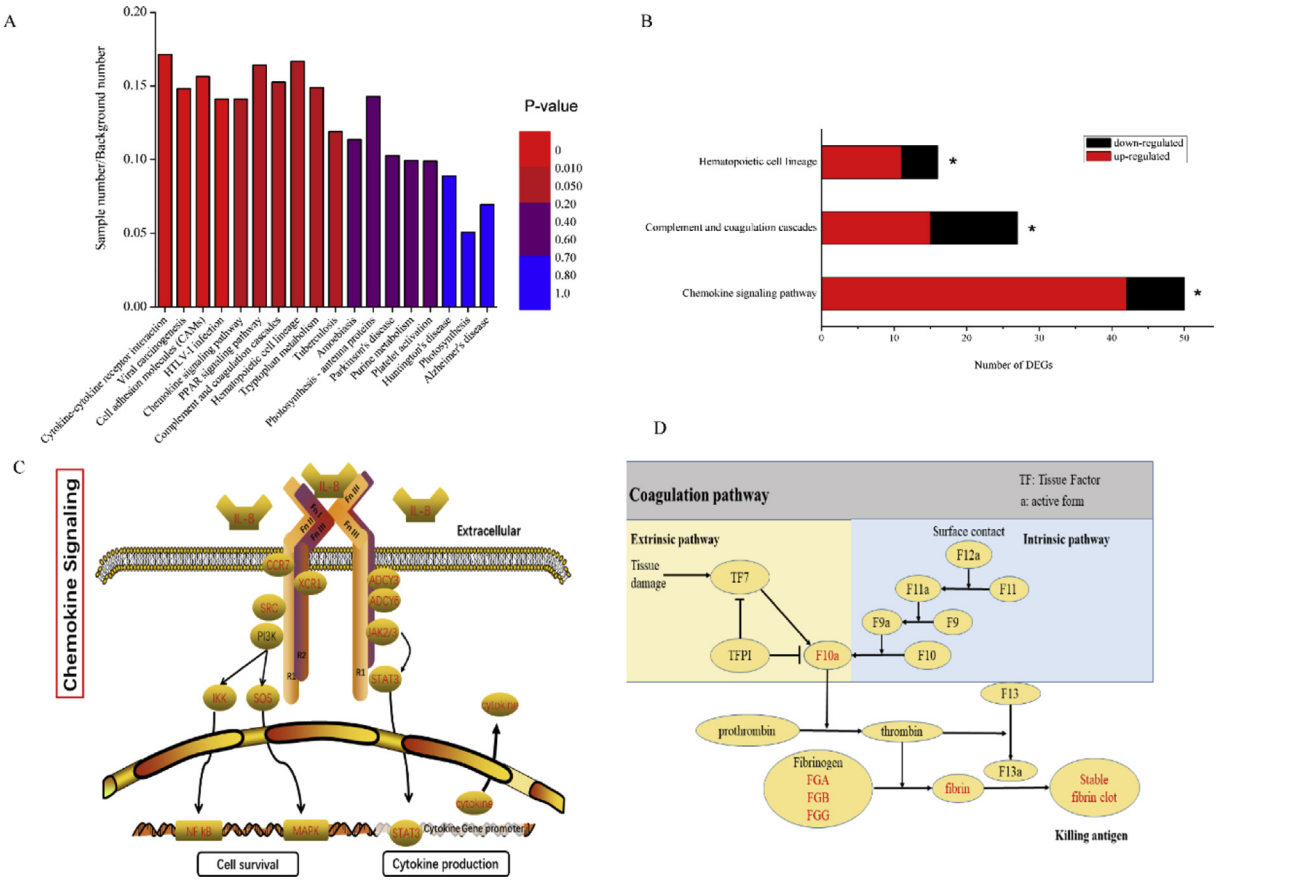

Fig. 4. Host enrichment kegg pathways including DEGs. A, DEGs involved in 18 kegg pathways. B, top3 significant enrichment keg pathways, red column denotes the number of significantly up-regulated genes; black, significantly down-regulated. C, D, chemokines signaling pathway and coagulation pathway, respectively. red word indicates significantly up-regulated expression; black, unchanged expression.

infected with *L321_23611*-RNAi strain of *P. plecoglossicida* (Figure 4A). Among the 18 KEGG pathways, 3 were directly related to immune systems, which were chemokine signaling pathway, complement and coagulation cascades and hematopoietic cell lineage, respectively (Fig. 4B). In chemokine signaling pathway (Figure 4C), genes encoding IL8, ADCY3, ADCY6, CCR7 and XCR1 were highly up-regulated. In complement and coagulation cascades (Figure 4D), genes encoding FGA, FGB, FGG, SERPINA1 were highly up-regulate. In hematopoietic cell lineage, genes encoding CD2, CD3, CD8, CD22 and HLA-DR were highly up-regulated.

3.5. Different expression genes (DEGs) in P. plecoglossicida

The different expression genes of *P. plecoglossicida* between infected spleens are showed in Fig. 5A. Of 4914 profiled mRNAs of *L321_23611*-RNAi strain-infected spleen, 73 were significantly altered in abundance in contrast with wild-type-infected spleen. In all of 73 DEGs, 3 were up-regulate and 70 were down-regulated. Based on the criteria mentioned above, all of the 70 suppressed DEGs were selected and presented in Figure 5B. 63 of the 70 down-regulated DEGs were successfully included in 291 enrichment GO terms.

Bacterial-type flagellum-dependent cell motility, one of the enrichment GO terms, was significant virulence-related system (Figure 6B). In terms of KEGG database, 46 of 70 down-regulated DEGs were enriched in 46 KEGG pathways (Fig. 6A). Compared with the wild-type strain, all of the 46 KEGG pathways were down-regulated in spleen infected with *L321_23611*-RNAi strain of *P. plecoglossicida* and 6 of them were significantly down-regulated according to the criteria of P-value < 0.5(Figure 6A). Among the 6 KEGG pathways, top 3 were directly related to virulent systems, which were flagellar assembly, biosynthesis of amino acids and lysine biosynthesis, respectively (Figure 6B). In flagellar assembly systems, genes encode FliC, FlgF, FlgI, MotA and MotB were significantly down-regulated (Figure 6C). In biosynthesis of amino acids systems, genes encode SerA, ArgC, TrpE, GlyA, E1.1.1.3, LysA, ArgD, PhhA, MetE and LeuA were significantly down-regulated. In lysine biosynthesis systems, genes encode LysA, ArgD, MurF and E1.1.1.3 were significantly down-regulated.

4. Discussion

Recently, the RNA interference (RNAi) screen has proven to be an effective program for characterizing biological functions by targeting specific genes of interest [33,34]. In present study, RNAi screen approach was used to design five shRNAs to silence the target gene *L321_23611* of *P. plecoglossicida,* respectively. The bacterial pCM130/tac-*L321*_23611-shRNA-911 strain (called as the *L321_23611*-RNAi strain) markedly reduced the content of *L321*_236111 mRNA in *P. plecoglossicida* with a maximal decrease of 89.2%. which indicated the efficiency and reliability of RNAi technology. In contrast with infection of wild-type strain, the infection of *L321_23611*-RNAi strain caused only 92% mortality of *E. coioides* and the onset time delay for 1 day. The result indicated that RNAi of *L321_23611* attenuate the virulence of *P. plecoglossicida*, which suggested *L321_23611* gene contibuted to the virulence of *P. plecoglossicida* to *E. coioides*.

Recent researches showed that pathogenic infection can result in great changes at pathogen and host transcriptome [3,35]. Moreover, a single virulence-related gene can also lead to pervasive changes at both host and pathogen transcriptome during infection [6]. In the present study, compared with wild type strain, RNAi of *L321_23611* in *P. plecoglossicida* also lead to a significant change in both host and pathogen

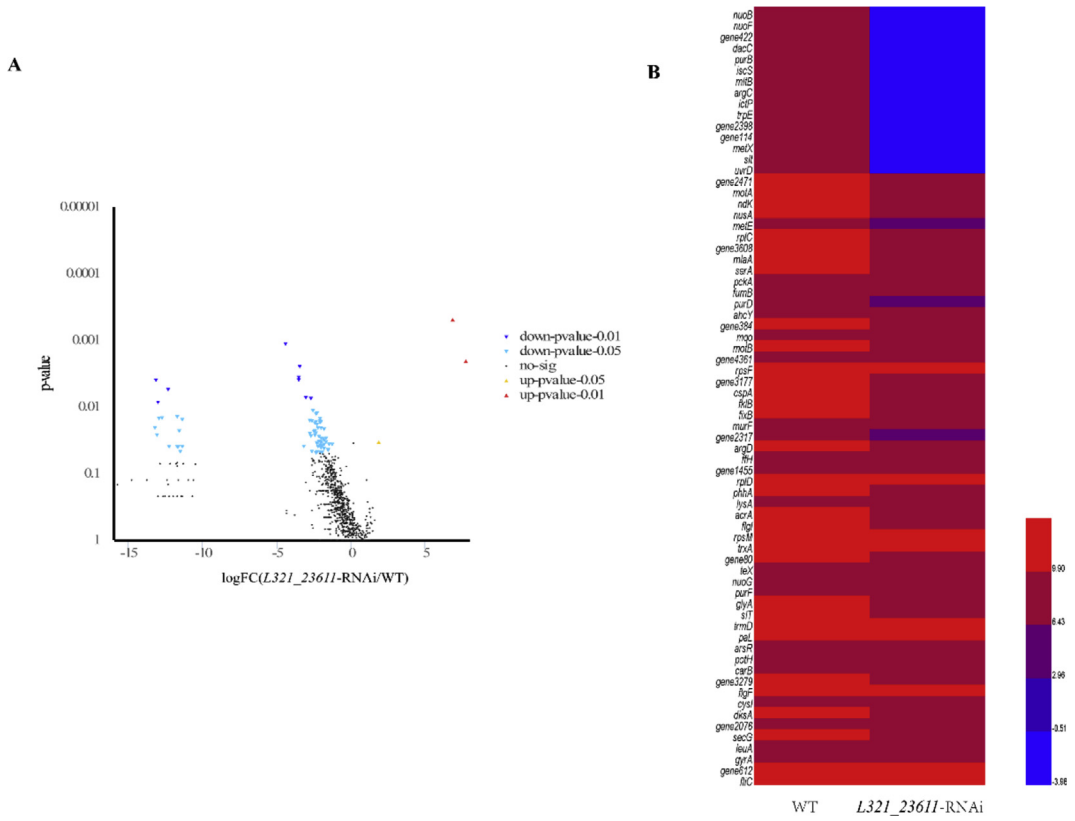

Fig. 5. The different expression genes (DEGs). A, volcano of DEGs. B, heat map of 70 depleted DEGs.

transcriptome. The results indicated the great impact of *L321_23611* in the host-pathogen interaction of *P. plecoglossicida* and *E. coioides*. GO and KEGG analysis from host response showed that hemoglobin complex GO term and chemokine signaling, complement and coagulation cascades and hematopoietic cell lineage pathway were significantly activated by *L321_23611*-RNAi strain of *P. plecoglossicida* during infection. When hemorrhage occurs in damaged tissues, hemoglobin exhibits either an anti-inflammatory feature or a restorative function for infiltrating tissue-resident macrophages, which play a critical role in innate immune surveillance against tissue-invading pathogens [36,37]. Hematopoiesis occurs in the bone marrow and hematopoietic stem cells (HSC) have the lifelong capacity to self-renew and give rise to innate-like B and T cells [38,39]. B-cells have proven to periodically reduce circulating parasites levels during infection and were required for murine long term survival [40]. Chemokines were primarily proven to be potent attractants for leukocytes such as neutrophils and monocytes, and were thus in large part regarded as the mediators of acute and chronic inflammatory responses [41–43], while they function as antimicrobial molecules and play a critical role in host defenses against pathogen challenge [44,45]. In consistent with gilthead seabream injected with non-replicative virus or heat-killed bacteria, chemokine expression was up-regulated by the viral particles in spleen [46]. In the *Streptococcus iniae* infection, the expression of novel chemokine RbCXC mRNA was markedly upregulated in the spleen of fish [47]. Moreover, a specific chemokine IL8, an important inflammatory mediator, is responsible for the migration and activation of neutrophils [48], which could kill microorganisms through phagocytosis and modulate the immune response by interacting with other immune cells [49]. The complement and coagulation cascades belong to two protein-related cascades in plasma that mainly function in host defense and hemostasis, respectively [50]. Complement, an integral part of the innate immune system, functions as the primary host defense barrier killing bacteria via assembly of the Membrane Attack Complex (MAC) [51–53]. Coagulation play an important role in the early innate immune response since it's factors can entrap bacteria inside clots, support inflammatory response and generate small antibacterial peptide [54,55]. Complement and coagulation cascades pathway was strongly activated in *grass carp* against *grass carp reovirus* or *Aeromonas hydrophila* infection [56,57]. All of these enrichment in GO and KEGG pathways indicate the increased immune response from *E. coioides* infected with *L321_23611*-RNAi strain compared with the counterpart of *P. plecoglossicida.*

Bacterial virulence genes play key roles in pathogen survival and dissemination by subverting or eluding host defenses during infection [1,6,58]. In this study, from the aspect of *P. plecoglossicida* transcriptome, GO and KEGG analysis showed that bacterial-type flagellum-dependent cell mortality GO term and flagellar assembly, biosynthesis of amino acids and lysine biosynthesis systems were significantly repressed by *L321_23611*-RNAi strain of *P. plecoglossicida* during infection. Motility driven by flagellar systems is essential for full pathogenesis for many bacteria, such as *P. aeruginosa, E. coli, Campylobacter jejuni*, *Vibrio cholera* [59,60]. However, the flagellum not merely contributed to virulence as a motility device, and it also played critical roles in many other infection processes such as adhesion, biofilm formation, effector molecule secretion and immune system modulation [61,62]. In *Trypanosoma brucei,* the assembly of the flagellum also showed a great contribution in host-parasite interactions, while flagellar membrane mediates its attachment to host tissues and harbours multiple virulence factors [63,64]. YplA, a virulence-associated phospholipase in the pathogenic bacterium *Yersinia enterocolitica*, was transported by the flagellar system, which could secrete type III protein to influence bacterial-host interactions [65]. For amino acid anabolism, lysine biosynthesis in *Aspergilli* has been investigated to be strictly required for pathogenicity [66]. A strong attenuation of virulence was observed under inhibition of fungal lysine biosynthesis [67–69]. Tabtoxin, derived from the lysine biosynthetic pathway, contributes significantly to *Pseudomonas syringae* virulence in plants [70]. All of these depletion of GO and KEGG pathways indicate the decreased virulence and invasion from the *L321_23611*-RNAi strain compared with the

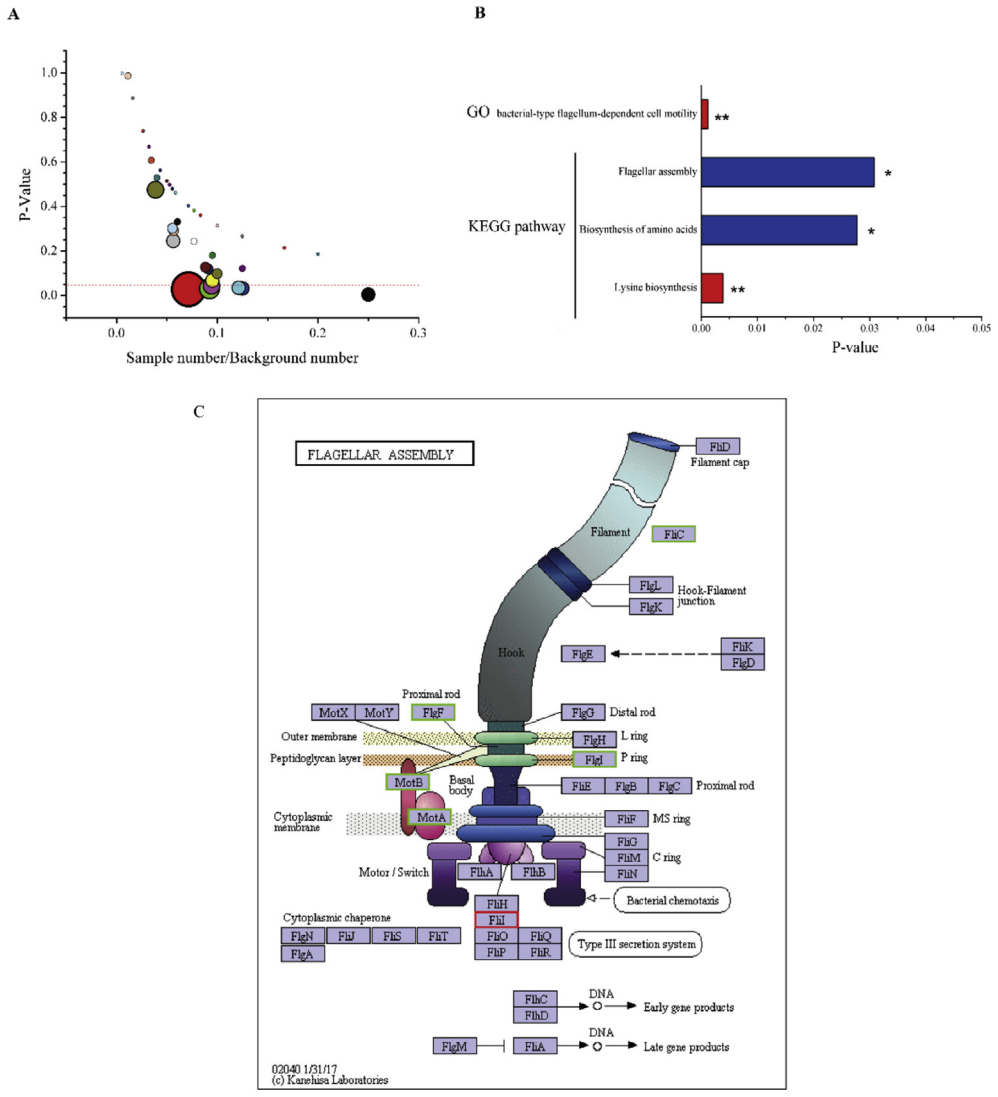

Figure 6. Pathogenic DEGs included in enrichment kegg pathways and GO term. A, 46 enrichment kegg pathways. B, top 3 significant enrichment kegg pathways and top 1 significant enrichment GO term. C, the details of flagellar assembly pathway, red contour indicates significantly up-regulated expression; blue, significantly down-regulated expression; black, unchanged expresssion according to $\log_2$FC(*L321_23611*-RNAi/WT).

counterpart of *P. plecoglossicida.* Although there has been researches about the response of both pathogen and host pathways to bacterial infection, no research has been found about the pathogen-host interaction to a single gene during infection. Both the down-regulated of these bacterial pathways and up-regulation of these host pathways in spleen infected by *L321_23611*-RNAi strain indicated that *E. coioides* was easier to kill *L321_23611*-RNAi strain than the wild type strain of *P. plecoglossicida*. The results also showed that the *L321_23611*-RNAi strain was less virulent and less abundant in *E. coioides*.

5. Conclusion

ABC transporter gene *L321_23611* was identified as a virulent gene of *P. plecoglossicida* by RNAi and infection experiment . Compared with the infection of wild type strain, infection of *E.coioides* with *L321_23611*-RNAi strain resulted in activation of genes in chemokine signaling pathway, coagulation and complement system, hematopoietic cell lineage pathway in *E. coioides* as well as hemoglobin complex GO term in spleen, and depression of bacterial genes in bacterial-type flagellum-dependent cell mortality GO term and flagellar assembly, biosynthesis of amino acids and lysine biosynthesis systems pathways in *P. plecoglossicida*.

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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.2019.05.046.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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