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Exploring the influence of the surface proteins on probiotic effects performed by *Lactobacillus pentosus* HC-2 using transcriptome analysis in *Litopenaeus vannamei* midgut

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ABSTRACT

In order to understand the mediation function of surface proteins in probiotic effects executed by *Lactobacillus pentosus* HC-2 in midgut of *Litopenaeus vannamei,* the immune and digestion related enzymes and the transcriptome expression were analyzed after shrimp fed with normal HC-2 or with stripped surface proteins HC-2 by lithium chloride (LiCl) treatment. The results showed that the shrimp fed with normal HC-2 produced much higher immune and digestion related enzymes than the control group or LiCl-treated HC-2 group to defense the *Vibrio parahaemolyticus* E1 infection. We obtained total over 275,099 unigenes from *L. vannamei* midgut, 981 genes were significant differentially expressed in normal HC-2 group compared with control, 1314 genes were significant differentially expressed in LiCl-treated HC-2 group compared with control, and 1689 genes were significant differentially expressed in LiCl-treated HC-2 group compared with normal HC-2 group. The GO/KEGG enrichment analysis of the significantly different genes demonstrated that *L. vannamei* fed with normal HC-2 induced immune-related, signal transduction, ion homeostasis, cell-cell adhesion, response stress/stimulus, vascular endothelial growth factor and peritrophin genes up-regulation, which were important genes involved in improving the shrimp intestine immune response, nutrition and growth performance, and bacteria adhesion and colonization, but these genes were suppressed in the midgut of shrimp fed with deprived surface proteins bacteria. Taken together, these results indicated that the surface proteins were essential for HC-2 executing probiotic effects in midgut of shrimp. Our data contribute to improve the current understanding of host - *Lactobacillus* interaction and the probiotic mechanisms in shrimps.

1. Introduction

Litopenaeus vannamei as an important commercially species is widely distributed over the world, which has become the most extensively cultured crustacean species [1,2]. Attract by the high nutritional value of *L. vannamei* and its increasing market demands, it became the dominant aquaculture species in China, since it was introduced in 1988. However, large-scale high-density shrimp aquaculture, overdose of bait and abuse of various antibiotics enhanced the incidence of disease outbreaks such as white spot syndrome virus (WSSV), Taura syndrome virus (TSV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV), and induced new drug-resistant pathogenic microorganisms [3,4], the shrimp culture industry is facing a series of challenges, which caused high mortality and serious economic losses [5,6].

Lactic acid bacteria (LAB) are a group of Gram positive bacteria and a potential probiotic microbe that produce lactic acid as a major or sole product from the fermentation of glucose [7,8]. They have the capacity to adhere to epithelial cells and mucus gel from the gastrointestinal tracts (GIT) of different species, which contributes to the health of the

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host based on their competitive adhesion and production of antagonistic substances against some pathogens bacteria [9,10]. In aquaculture, Lactobacillus was used to defend against the intestinal diseases mainly include *Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus acidophilus, Lactobacillus pentosus* and *C. inhibens,* especially the *L. plantarum* is mostly widely used in shrimp breeding [11–14]. The intestine is the main organ of shrimp which performs many administrative functions including synthesis and secretion of immune or digestive enzymes, absorption of nutrients, storage of reserves, and excretion [15]. Several studies reported that the dietary *Lactobacillus* can improve the intestinal morphology, such as increasing the length of intestinal villi and the absorption area of nutrients [16]. LAB also can secrete extracellular digestive enzymes or stimulate the host cells to produce digestive enzyme such as protease, lipid enzyme and carbohydrate, helping the body to strengthen the digestion of nutrients [17]. Moreover, the metabolism of the LAB can produce nutrients, such as amino acids, vitamins and proteins [18,19].

Adhesion is a specific interaction between the *Lactobacillus* bacterial surface components and the host cell surface, which is a crucial and prerequisite step for its colonization in the digestive tract [20], and the surface proteins have been proposed to be involved in colonization of gastrointestinal epithelial cells and mucosa of mammals [21]. Surface layers (S-layers) are monomolecular crystalline arrays composed of protein or glycoprotein subunits with molecular masses ranging from 40 to 200 kDa [22]. S-layer proteins represent 10–15% of the total protein of the bacterial cell indicating efficient gene expression, S-layer protein synthesis and secretion [23,24]. These proteins bind to the outermost layer of the cell with non-covalent bonds, using denaturants lithium chloride (LiCl), guanidine hydrochloride (GuHCl), urea or metal chelating agents etc can depolymerize them to monomer [25–27]. Actually, except the adhesive capacity, surface proteins also have the ability of maintaining the shape of the bacteria, molecular sieve function, immunomodulation to the host, and providing extracellular enzyme binding sites [28–30]. Previous studies showed that *L. pentosus* HC-2 is a probiotic strain that can enhance the immune responses, growth performance and disease resistance of the *L. vannamei* [14], but the effects of the surface proteins of HC-2 on the intestines of *L. vannamei* are still poorly understood at the molecular level. Therefore, to further understand the role of the surface proteins in the process of colonization and immune regulation of *L. pentosus* HC-2 in intestine of *L. vannamei*, and the intestinal reaction of *L. vannamei* to antigen (dietary, microbial, or both) of the cell-mediated type, and to evaluate the effects of different diets on the shrimp immunomodulation, a fundamental knowledge of the surface proteins of HC-2 on the shrimp intestinal physiology is required.

In this study, we detected the immune and digestion related enzyme levels and performed a transcriptomic analysis in the midgut of *L. vannamei* fed with normal HC-2 or LiCl-treated *L. pentosus* HC-2. The newly identified genes activated by surface proteins of HC-2 can provide a better insight into understanding the interaction between *Lactobacillus* and *L. vannamei.*

2. Materials and methods

2.1. Preparation of experimental diets

Lactobacillus pentosus HC-2 was isolated from the gut of healthy fish (*Chaeturichthys stigmatias*) previously by our laboratory saved in de Man, Rogosa, and Sharpe (MRS) broth (Qingdao Hope Biol-Technology Co., Ltd., Qingdao, China) containing 15% sterile glycerol solution and stored at −80 °C [14]. *L. pentosus* HC-2 was grown in MRS broth at 37 °C under anaerobic conditions for 18 h and prepared as a fermentation seed culture. For the negative control diet, the cell pellet from 500 ml of culture were harvested by centrifugation (3000 × *g*, 10 min, 4 °C) and washed three times with sterilized seawater. Subsequently, cells were resuspended in sterilized seawater and sprayed on commercial basic feed at 5×10^8 CFU g feed^{-1}. For the experimental diet, cultures bacterial strains were harvested and incubated the cells in 5 M LiCl [31]. After treatments, cells pellet were harvested by centrifugation (3000 × *g*, 10 min, 4 °C) and washed three times with sterilized seawater and resuspended in sterilized seawater and sprayed on commercial basic feed at 5×10^8 CFU g feed^{-1}. Feed was stored at 4 °C after dried at room temperature for 5 h, and the feed was prepared every week.

2.2. Shrimp and diet experiment

Experimental shrimp (3.0 ± 0.20 g) were obtained from Ruizi Seafood Development Co. Ltd. in Qingdao (Shandong, China). A total of 900 healthy shrimp were randomly divided into 9 aquaria (600 L), each aquaria containing 100 shrimp. Three groups: C group, fed with the commercial basic diet; R group, fed with the commercial basic diet + normal HC-2; L group, fed with the commercial basic diet + LiCl-treated HC-2, and each diet treatment was conducted with triplicate experiments. Shrimp were maintained in fresh seawater (salinity, 30‰; temperature, 30 ± 2 °C) with continuous aeration. During the experiment period, shrimp were fed three times per day (at 7:00, 11:00 and 19:00) for 4 consecutive weeks, and daily feeding rate was 10% of the body weight. To guarantee water quality, remaining feed and faeces were removed by siphon every morning before feeding and replace the 50% water. Shrimp were acclimatized for one week with the basical diet before the experiments.

2.3. RNA isolation and cDNA library construction

After four weeks feeding, the midguts from 20 shrimp of each group were taken by sterile forceps and rinsed by sterile PBS, and stored in liquid nitrogen (- 196 °C) until RNA isolation. Total RNA was extracted using TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) and RNeasy Mini Kit (Qiagen Corp., Hilden, Germany) according to the manufacturer's instructions. The quantity and integrity of RNA were assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, Palo Alto, CA, USA). A total of 3 mg of high-quality RNA per group were used for cDNA synthesis and sequencing.

Sequencing libraries were prepared using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. The mRNA was fragmented into small pieces (200–300 bp) through ions. RNA fragments were reverse transcribed into first-strand cDNA using M-MuLV Reverse Transcriptase with random hexamer primers. Second-strand cDNA synthesis was then carried out using DNA Polymerase I and RNase H, and the dTTP is replaced by dUTP to achieve the purpose of chain specific library. These cDNA fragments underwent end-repair and adapter ligation. The resulting products were purified and PCR-amplified to create the final cDNA libraries. The size of the library was evaluated by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), and the concentration of the library was detected by fluorescence quantification prior to emulsion PCR and sequencing as recommended by Roche.

2.4. Illumina sequencing, gene assembly and annotation

Transcriptome sequencing was carried out on an Illumina HiSeq 4000 platform that generated ~200 bp pair-end raw reads (Novogene Bioinformatics Technology Co. Ltd., Beijing, China). Before proceeding to de novo assembly, the raw reads were first trimmed low quality reads (with quality scores lower than 20) and removed adaptor sequences by Trimmomatic tool, ambiguous 'N' nucleotides (with a 'N' ratio over 10%). De novo assembly was performed by the Trinity program [32], followed by cd-hit-est tool clustering nucleotide sequences with default parameters. Non-redundant unigene sequences were used for sequence

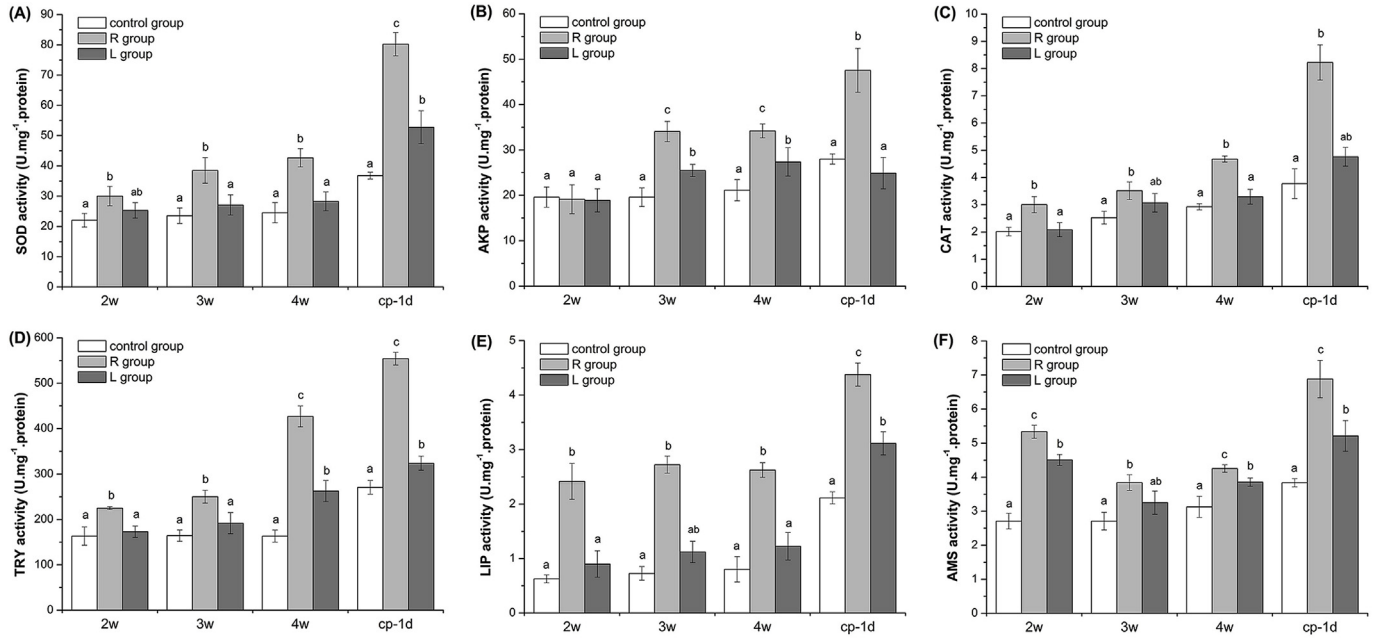


Fig. 1. Immune and digestion related enzyme activities in midguts of *L. vannamei* during the experimental period. A–F: (Superoxide Dismutase, SOD), (Alkaline phosphatase, AKP), (Catalase, CAT), (Trypsin, TRY), (Lipase, LIP), (α-Amylase, AMS). Mean values and standard deviation (± SD) are presented for each parameter (n = 10). Means in the same row with different letters are significantly different ($P < 0.05$). Shrimp were fed a basic commercial diet (C), or supplemented with *L. pentosus* HC-2 (R), or supplemented with LiCl-treated *L. pentosus* HC-2 (L). Note, the abscissa represent time at 2, 3 and 4 weeks after the experimental feeding, and cp-1d means at one day post challenge.

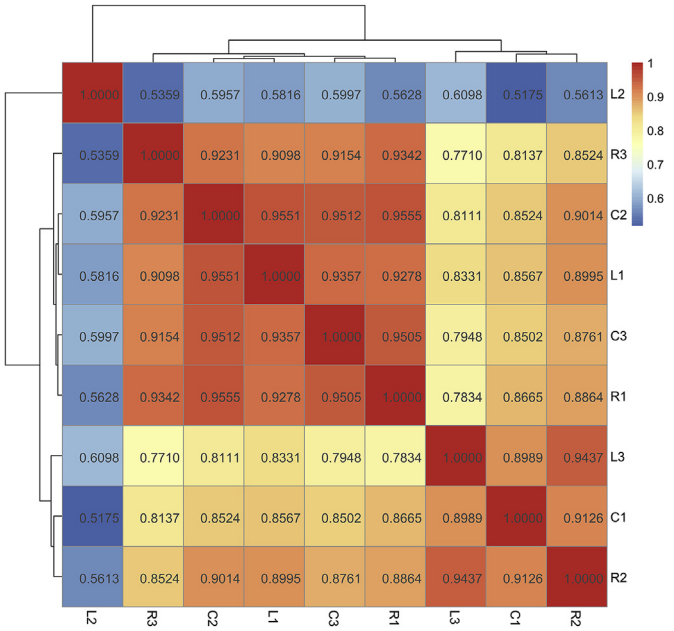


Fig. 2. Pearson correlation coefficients between samples. Shrimp were fed a basal diet (C [C1, C2, C3]), or supplemented with *L. pentosus* HC-2 (R [R1, R2, R3]), or supplemented with LiCl-treated *L. pentosus* HC-2 (L [L1, L2, L3]).

annotation using the databases Gene Ontology (GO) annotation and Pathway assignment based on the kyoto encyclopedia of genes and genomes (KEGG) database (http://www.genome.jp/kegg). Protein family, NCBI (http://www.ncbi.nlm.nih.gov/), non-redundant protein database (Nr), Evolutionary genealogy of genes: Non-supervised Orthologous Groups (http://eggnogdb.embl.de/) and Swiss-Prot [33–36].

Table 1
Summary of the annotations of *Litopenaeus vannamei* unigenes.

Database	Number	Percentage
NR	27286	9.92
GO	9823	3.57
KEGG	5452	1.98
eggNOG	24122	8.77
Swissprot	19241	6.99
In all database	3028	1.10

2.5. Identification of differentially expressed genes (DEGs)

The raw PE reads were mapped to cluster transcripts using Burrows-Wheeler Aligner (BWA) (http://bio-bwa.sourceforge.net/). For transcript abundance estimation, the value Fragments Per Kilobase of transcript per Million mapped reads (FPKM) was calculated by eXpress (http://bio.math.berkeley.edu/eXpress). The DEGseq method of the R statistical package was used to analysis the differential gene expression [37]. The P-value was adjusted using the q-value [38]. The q-value < 0.05 and $\log_2|\text{fold change}| > 2$ were as threshold for significantly different expression levels. GO functional enrichment assessment were performed to identify the biological function of differentially expressed genes. GO functional enrichment analysis and KEGG pathway enrichment assessment were used GOstats (https://bioconductor.org/packages/release/bioc/html/GOstats.html) with default parameters.

2.6. Validation of the RNA-seq profiles by quantitative real-time PCR (qPCR)

In order to validate the transcriptome data, 8 differentially expressed *L. vannamei* genes were selected to quantify their relative expression by quantitative real-time PCR (qRT-PCR). We randomly sampled ten shrimp from each group and extracted the RNA from the midguts by using TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) and RNeasy Mini Kit (Qiagen Corp., Hilden, Germany). The first-strand cDNA was synthesized using 2 μg RNA for

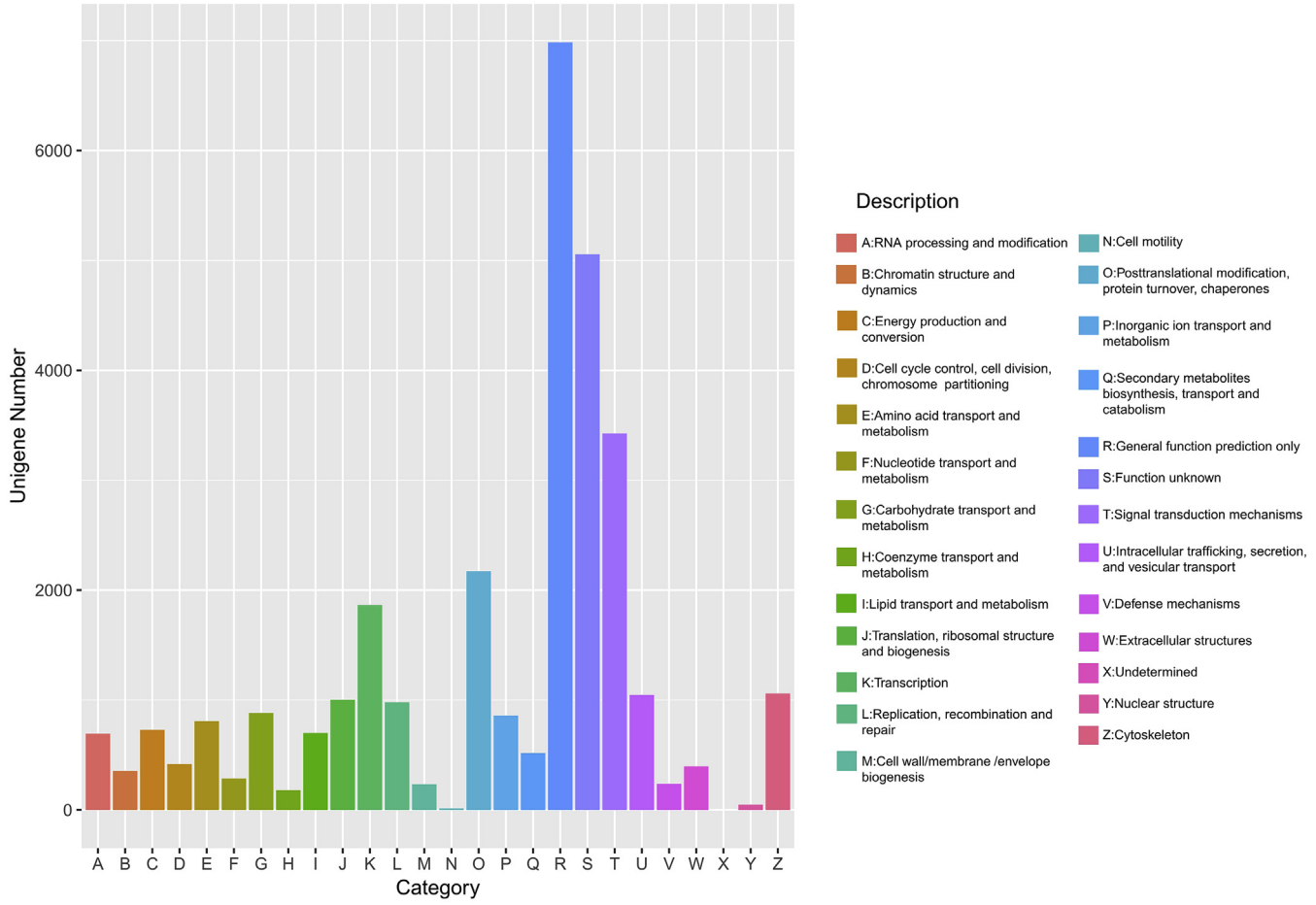


Fig. 3. Histogram presentation of eggNog Orthologous Groups (eggNOG) classification of 24,122 known protein-annotated unigenes. Each bar represents the number of unigenes classified into each of the 26 eggNOG functional categories.

each reaction by TransScript® One-Step gDNA Removal and cDNA Synthesis Kit (TransGen Biotech Co., Ltd., Beijing, China). The specific primer pairs (S1Table 1) were designed to measure the relative genes expression leves, and *β*-actin gene was as the endogenous control. PCR amplification was performed using the LineGene K Real-Time PCR System (BIOER, Hangzhou, China), and the TransStar Top Green qPCR Super mix (TransGen Biotech Co., Ltd.) following the manufacturer's recommendations. Each sample was run in triplicate. Each qPCR was performed in a total volume of 20 μL containing 10 μL of SYBR Green I Master, 0.4 μL of forward and reverse primers (10 mM), 2 μL of diluted cDNA and RNase-free water. PCR amplification was performed using the following cycling conditions: denaturation for 30 s at 94 °C, followed by 40 cycles of 5 s at 94 °C, and 30 s at 60 °C. The identities of all the products were confirmed by sequencing.

2.7. Challenge tests

The pathogenic strains of *Vibrio parahaemolyticus* E1 ATCC 17802 for the bacterial infection was donated by Doctor Wenbin Zhan (Laboratory of Pathology and Immunology of Aquatic Animals, Ocean University of China) saved in trypticase soy broth (TSB) containing 15% sterile glycerol solution and stored at −80 °C. *V. parahaemolyticus* E1 was cultured aerobically in 2216E broth (Qingdao Hope Biol-Technology Co., Ltd) at 28 °C for 18 h. After the breeding experiment, 25 shrimp from each aquarium with three replicates were challenged for three days in a tank with 30 L of seawater containing 10^7 CFU/mL live *V. parahaemolyticus* E1 and were fed with basical diet.

2.8. Immune and digestion related enzyme activity assay

The activities of several important immune and digestion enzymes including antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and Alkaline phosphatase (AKP), and digestion enzymes of Trypsin (TRY), Lipase (LIP) and α-Amylase (AMS) in the midgut of *L. vannamei* were determined at the 2-, 3-, 4- weeks post feeding experiment and at the first day after challenged. Ten shrimp were freely selected to sample the midguts and saved in liquid nitrogen, then add the tissue extract and grind the tissue to pieces [17]. Collecting supernatant to detect the enzymes activities using commercial colorimetric kits (Nanjing Jiancheng Bioengineering Research Institute) according to the operation manual.

2.9. Statistical analysis

The qRT-PCR data were analyzed using MX Pro-Mx3000P Multiplex Quantitative PCR system Software and the relative expression ratio (R) of mRNA was calculated according to the formula $2^{-\triangle\triangle Ct}$ [39]. Data are presented as mean ± standard deviation. All the data were analyzed using SPSS 19.0 (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant.

3. Results

3.1. Immune and digestion related enzyme activity

To investigate the effects of surface proteins on the immune and

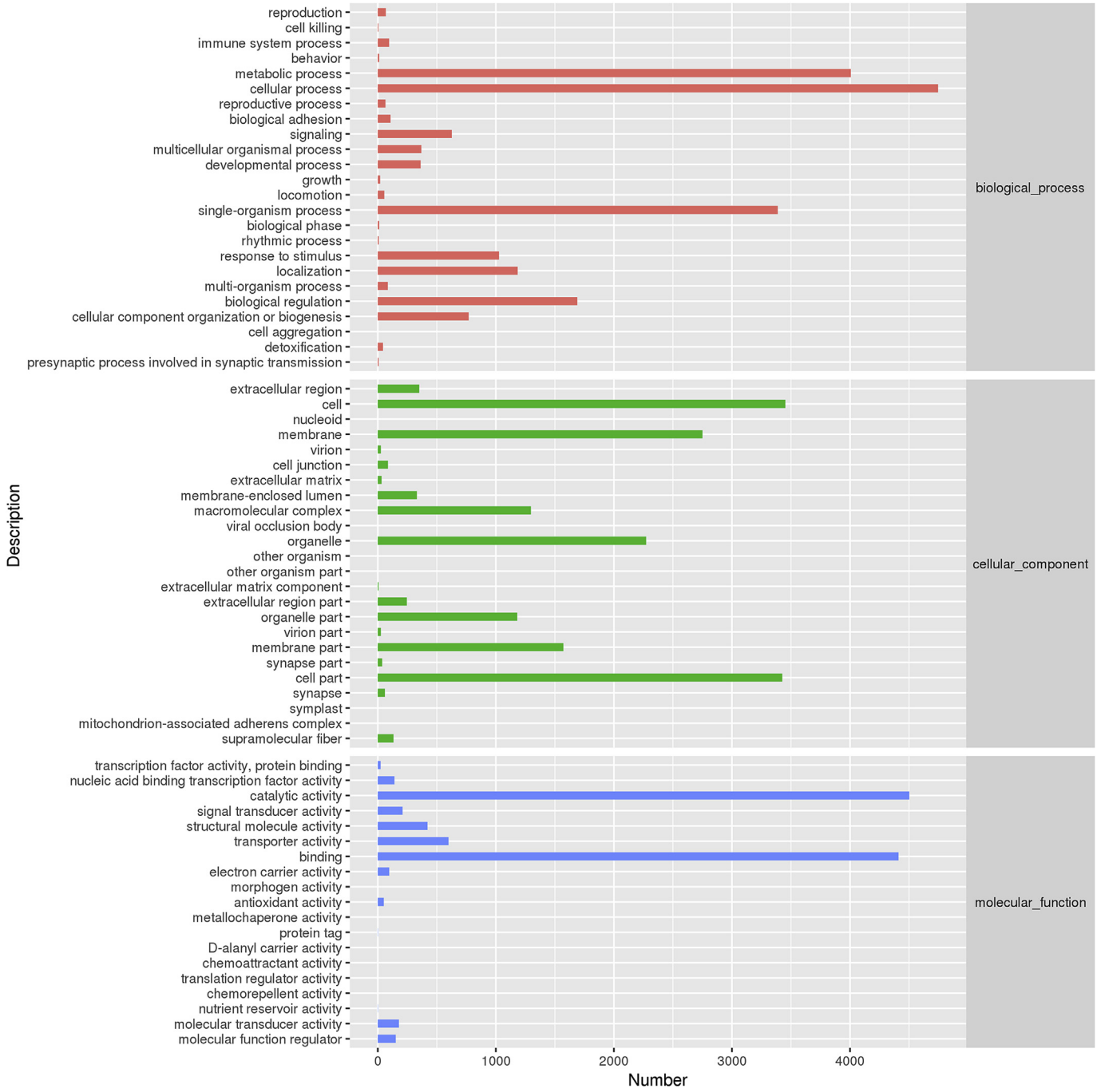


Fig. 4. Gene ontology (GO) classification of the total protein-annotated unigenes. Unigenes sequences were systematically classified into the GO sub-categories of the gene ontology catalogue system: biological process, cellular component and molecular function. Each bar represents the relative abundance of unigenes classified under each subcategory.

digestion related enzyme activities, we determined the activities of six important immune and digestion enzymes including antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), Alkaline phosphatase (AKP), and digestion enzymes of Trypsin (TRY), Lipase (LIP) and α-Amylase (AMS) in the midgut of *L. vannamei* as shown in Fig. 1. At two weeks after feeding, the activities of SOD, CAT, TRY, LIP and AMS were significantly higher in R group than in the C group or L group. The activities of SOD, CAT and TRY were display increase tendency from two to four weeks in R group, and the activities of AKP, CAT and TRY were increasing from 2 to 4 weeks in the L group, but the activity of AMS were decreasing from two weeks to three weeks in R group and L group. Additionally, the activities of the six enzymes were keep stable in the control group. However, the activities of the six enzymes were increased among the three groups post the shrimp were challenged by the *V. parahaemolyticus* E1, and the activities level of the six enzymes were significantly higher in R group than in C group or L group, and the SOD, TRY, LIP and AMS activities were significantly higher in L group than in C group.

3.2. Transcriptome sequencing and sequence assembly

In order to identify the genes involved in process of adhesion, colonization or probiotic functions performed by the surface proteins of HC-2, we created nine cDNA libraries from mRNAs extracted from the

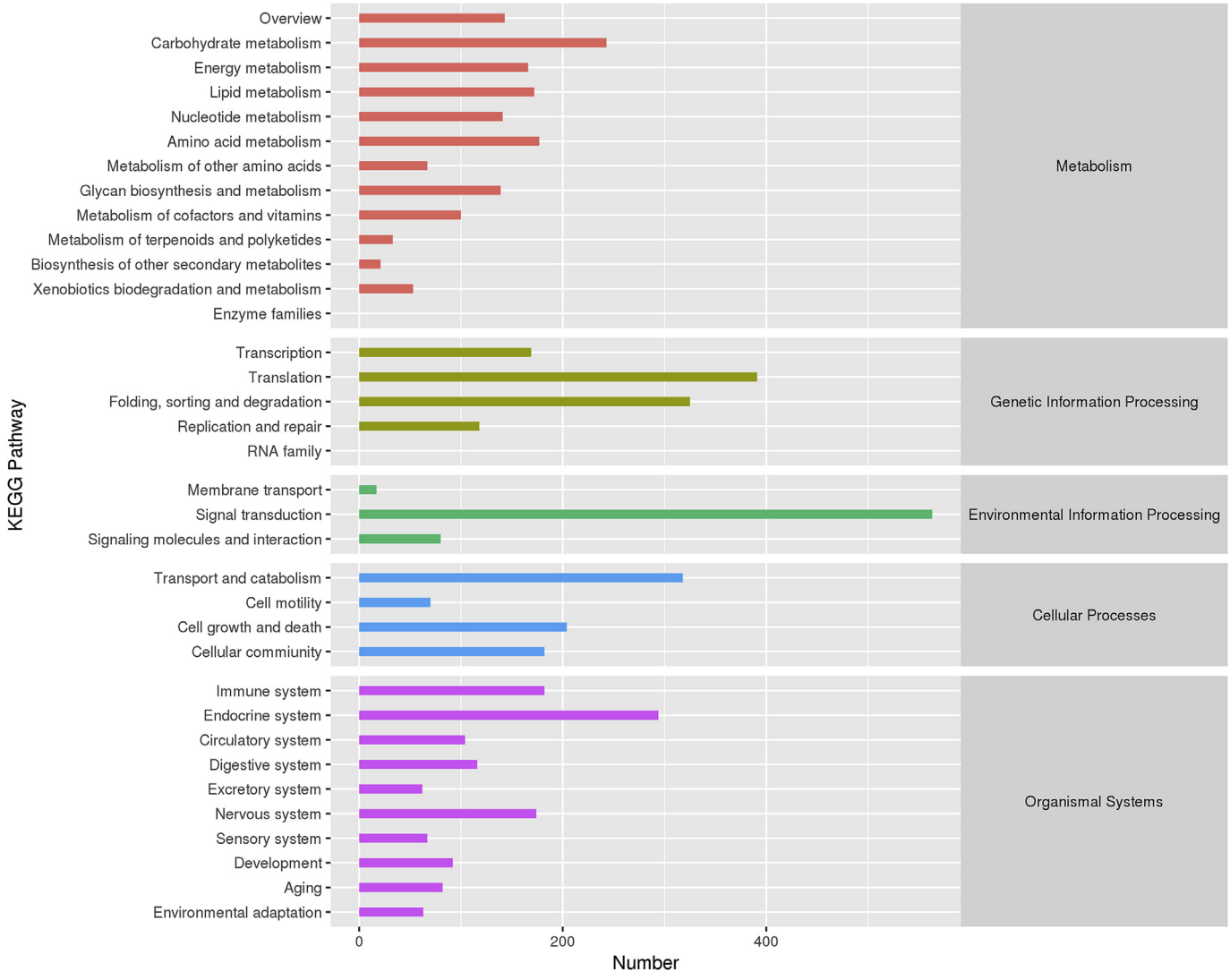


Fig. 5. KEGG biological pathway classification histograms for annotated unigenes.

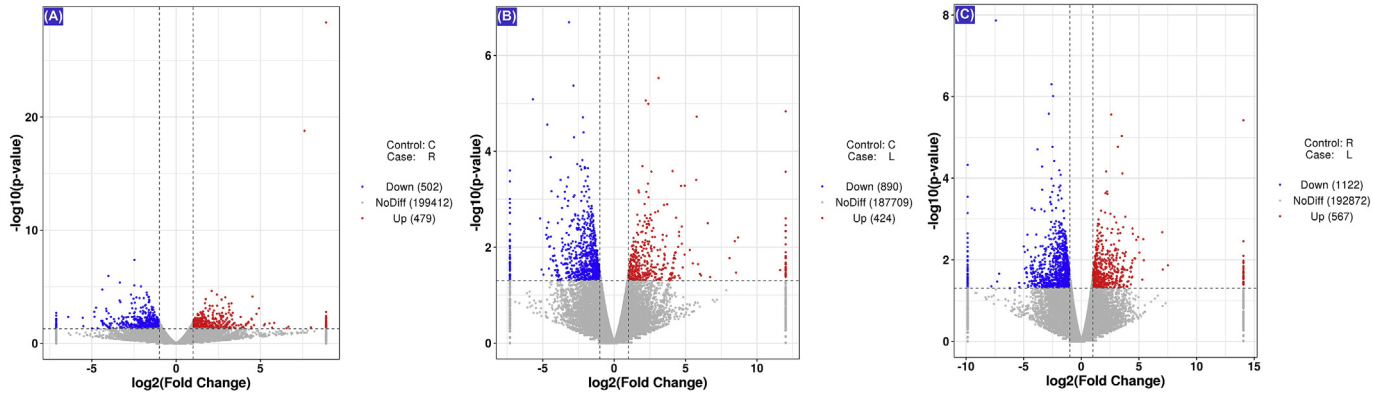


Fig. 6. Volcano plot of differentially expressed genes in shrimp fed a basic commercial diet (C) or a basic commercial supplemented with *L. pentosus* HC-2 (R), a basic commercial supplemented with LiCl-treated *L. pentosus* HC-2 (L). Differentially expressed genes were selected by q-value < 0.05, and $\log_2$ |fold change| > 2. The x-axis shows the fold change in gene expression among C, R and L groups, and the y-axis shows the statistical significance of the differences. Splashes represent different genes. Red splashes represent significantly up-regulated genes. Blue splashes represent significantly down-regulated genes. Shaded area splashes represent genes without significant differences in expression. The corrected P-value is represented by -log10 (p-adjusted). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

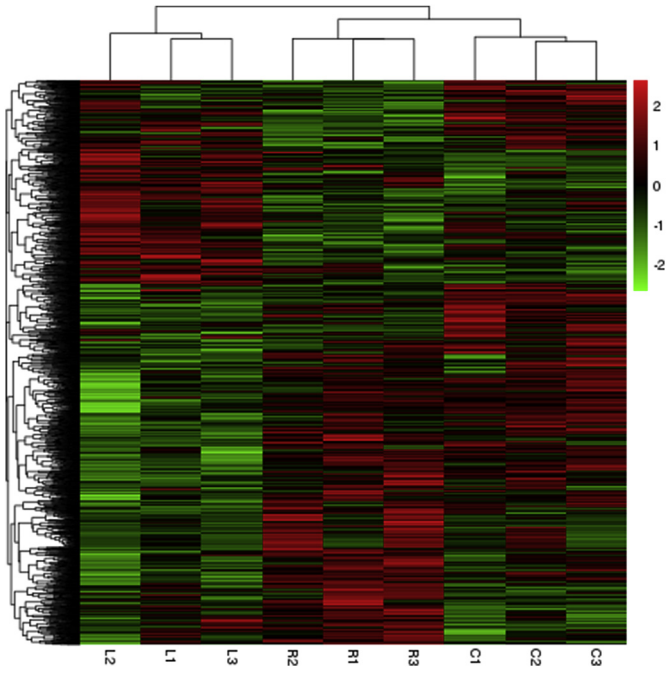


Fig. 7. Heat map of genes cluster analysis in the 9 samples of shrimp. Shrimp were fed a basal diet (C [C1, C2, C3]), or supplemented with *L. pentosus* HC-2 (R [R1, R2, R3]), or supplemented with LiCl-treated *L. pentosus* HC-2 (L [L1, L2, L3]).

mid-gut of shrimp fed with basal diet, or supplemented with normal HC or with LiCl-treated HC-2. For each group, high-throughput RNA sequencing resulted in 40.66–46.42 million single-ended reads (S1Table 2). After filtering for low quality reads, and for adapter sequences, 40.46–46.16 million clean reads (96.21–96.69% from raw data) were generated. From the clean data of the nine libraries, about 99.19–99.29% of clean sequences were successfully mapped to the entire reference transcriptome (S1Table 2). The overall assembly was performed using the combined clean reads from the nine libraries. De novo assembly using the Trinity software produced 275,099 unigenes (including contigs and singletons ranging from 201 to 18,575 bp), with an average length of 584 bp. The length distribution of unigenes is shown in S1Fig. 1.

Pearson correlation coefficient between replicates was high, with an average r coefficient 0.88 for control shrimp, 0.89 for shrimp fed with normal HC-2, and 0.67 for shrimp fed with LiCl-treated HC-2 (Fig. 2). These values indicate a satisfactory reproducibility of the biological replicates.

3.3. Functional gene annotation and classification

To annotate assembled unigenes, we performed BLAST against the genetic databases NR (27,286 genes were annotated), GO (9823 genes were annotated), KEGG (5452 genes were annotated), eggNOG (24,122 genes were annotated), and Swiss-Prot (19,241 genes were annotated) (Table 1).

In order to classify the genes and to predict their functions, standard unigenes were analyzed using the eggNOG database. According to eggNOG annotation, the unigenes were functionally classified into 26 protein families, apart from the undefined functional classes (ie, function unknown and general function prediction only), the other mainly involved in Signal transduction mechanisms Posttranslational modification, Protein turnover, Chaperones, Transcription, Cytoskeleton, Intracellular trafficking, Secretion, Vesicular transport, Translation, ribosomal structure, Biogenesis (Fig. 3).

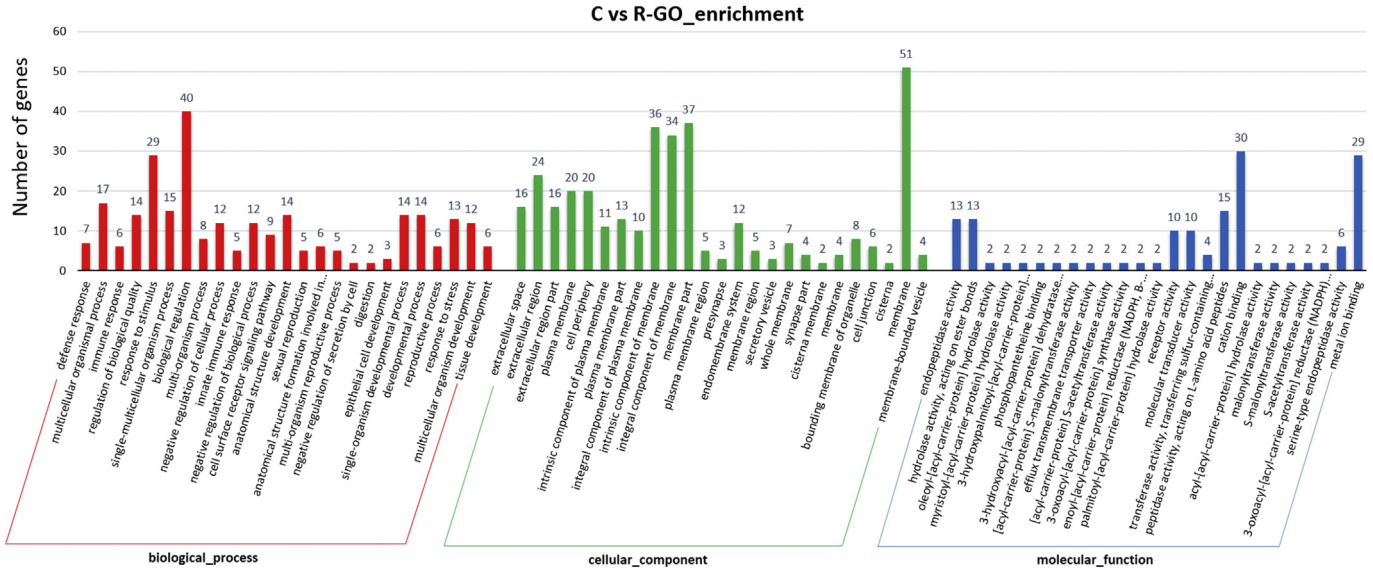
GO annotation was used to classify the transcripts into functional groups according to the GO category. In the biological process category, the most represented genes were related to metabolic process, cellular process, single-organism processes, biological regulation, localization, response to stimulus and cellular component organization. In the cellular component category, the most represented genes were related to the cell, cell parts, membrane, membrane part, organelle, organelle part, and macromolecular complex. In the molecular function category, the most represented genes were related to catalytic activity, binding, transporter activities and structural molecule activity (Fig. 4).

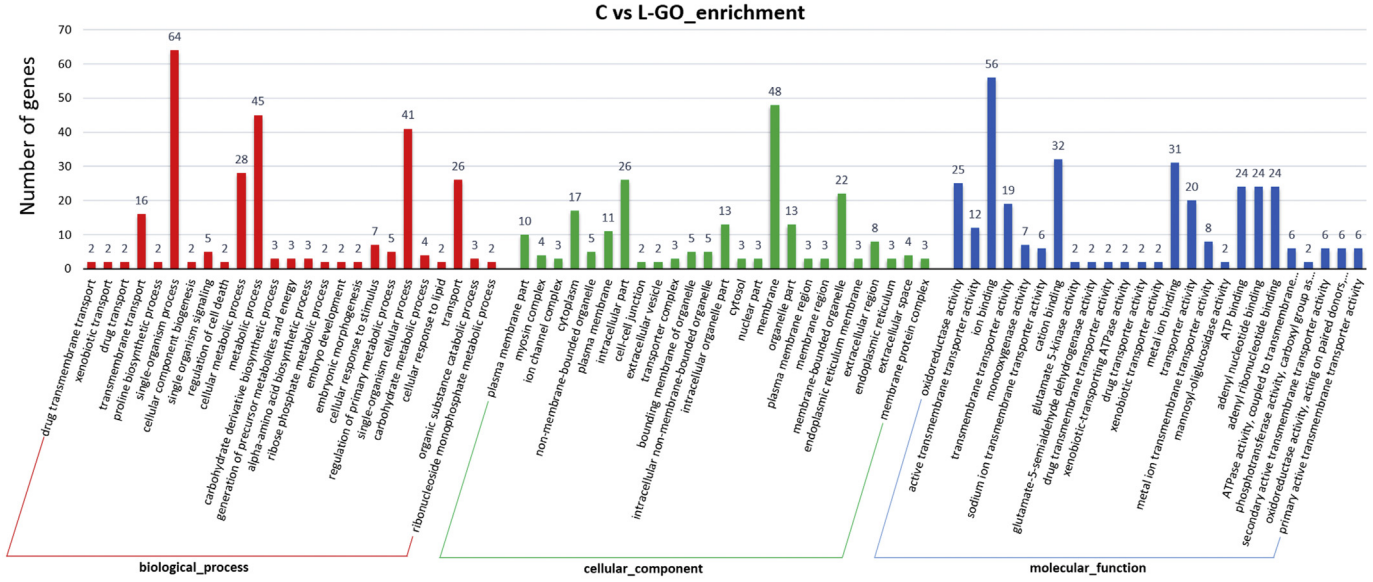
Pathway-based analysis make it available to have an indepth understanding the biological functions and gene interactions. The KEGG Pathway database is a collection of pathway maps drawn manually, representing the current knowledge on molecular interactions and reaction networks. Using the KEGG database, 5128 unigenes were grouped into 34 pathways. The majority of the unigenes fell into the categories of Metabolism (1,455), Genetic Information Processing (1,003), Environmental Information Processing (660), Cellular Processes (774) and Organismal Systems (1,236) (Fig. 5).

3.4. Identification of DEGs

DEGs were selected by DESeq if the $|\log_2$fold change$|$ was > 2 and their P-value was < 0.05. The volcano plot showing the number and relationship between fold-change and P-value of up or down-regulated DEGs in each comparison are shown in Fig. 6. R group was compared with the control group, a total of 199,412 unigenes were no difference; 479 of these were significantly up-regulated, whereas 502 unigenes were significantly down-regulated. L group was compared with the control group, a total of 187,709 unigenes were no difference; 890 of these were significantly up-regulated, whereas 424 unigenes were significantly down-regulated. L group was compared with the R group, a total of 192,872 unigenes were no difference; 567 of these were significantly up-regulated, whereas 1122 unigenes were significantly down-regulated. Cluster analysis was used to determine the expression patterns of differentially expressed genes under different experimental conditions (Fig. 7). Heat map showed that samples of C, R and L groups were clustered respectively, indicating that the genes expression in C, R and L groups differed and that the genes expression pattern in the midgut was influenced by the addition of probiotics or LiCl-treated probiotics.

GO functional enrichment analysis was performed to identify the biological function of DEGs ($\log_2$ $|$fold change$|$ > 2, q-value < 0.05) in shrimp fed with HC-2 or LiCl-treated HC-2 (Fig. 8). The results of the GO enrichment analysis of R-vs-C showed that up-regulated DEGs were mostly enriched in the following categories: BP (biological regulation, response to stimulus, multicellular organismal process, regulation of biological quality, single-multicellular organism process, negative regulation of cellular process, negative regulation of biological process, anatomical structure development, cell surface receptor signaling pathway), CC (membrane part, integral component of membrane, intrinsic component of membrane, plasma membrane, cell periphery), BP (endopeptidase activity, hydrolase activity, acting on ester bonds, receptor activity, molecular transducer activity, cation binding). The results of the GO enrichment analysis of L-vs-C showed that DEGs were mostly enriched in BP (single-organism process, transmembrane transport, cellular metabolic process, metabolic process), CC (membrane, intracellular part, cytoplasm, intracellular organelle part, plasma membrane, organelle part), MF (ion binding, oxidoreductase activity, cation binding, transmembrane transporter activity, active transmembrane transporter activity, metal ion binding, transporter activity). The results of the GO enrichment analysis of L-vs-R showed that DEGs were mostly enriched in the following categories: BP (establishment of localization, single-organism localization, single-organism transport, transport, metal ion transport, epithelium development), CC (intrinsic component of membrane, integral component of membrane, membrane part, plasma membrane, cell periphery, plasma membrane part, integral





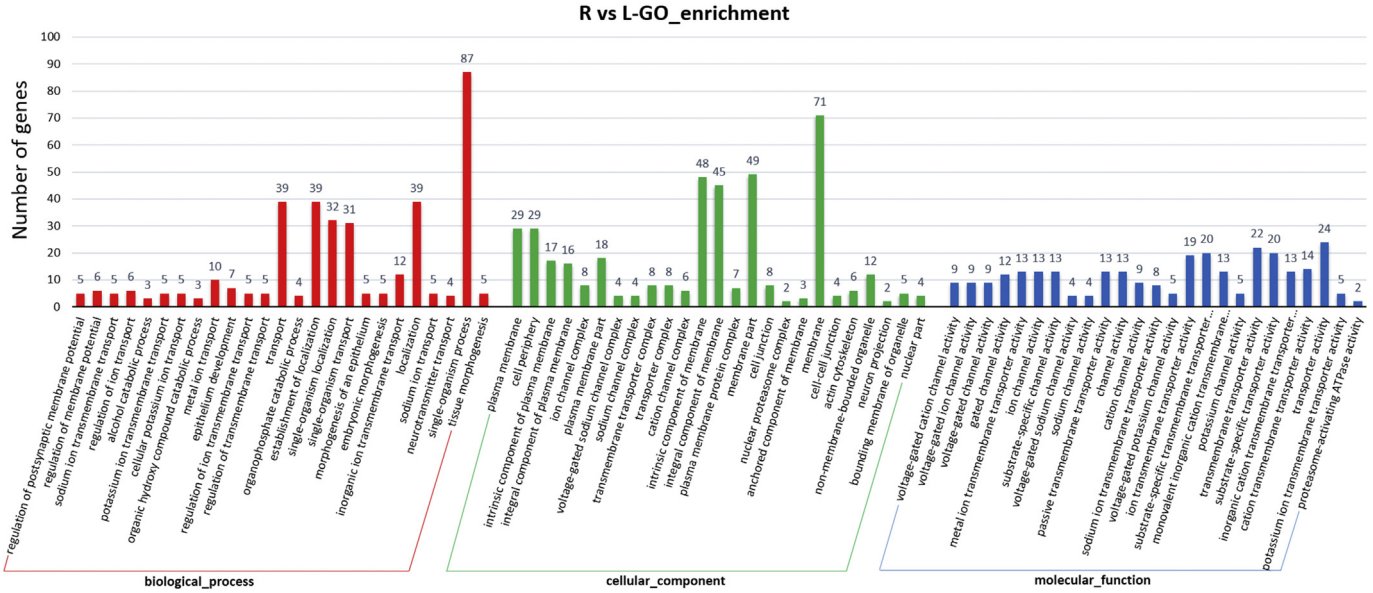


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Fig. 8. GO enrichment analysis of differentially expressed genes in shrimp midgut. BP: Biological Process, MF: Molecular Function, CC: Cellular Component. C: shrimp were fed a basic commercial diet; R: shrimp were fed a basic commercial supplemented with *L. pentosus* HC-2; L: shrimp were fed a basic commercial supplemented with LiCl-treated *L. pentosus* HC-2.

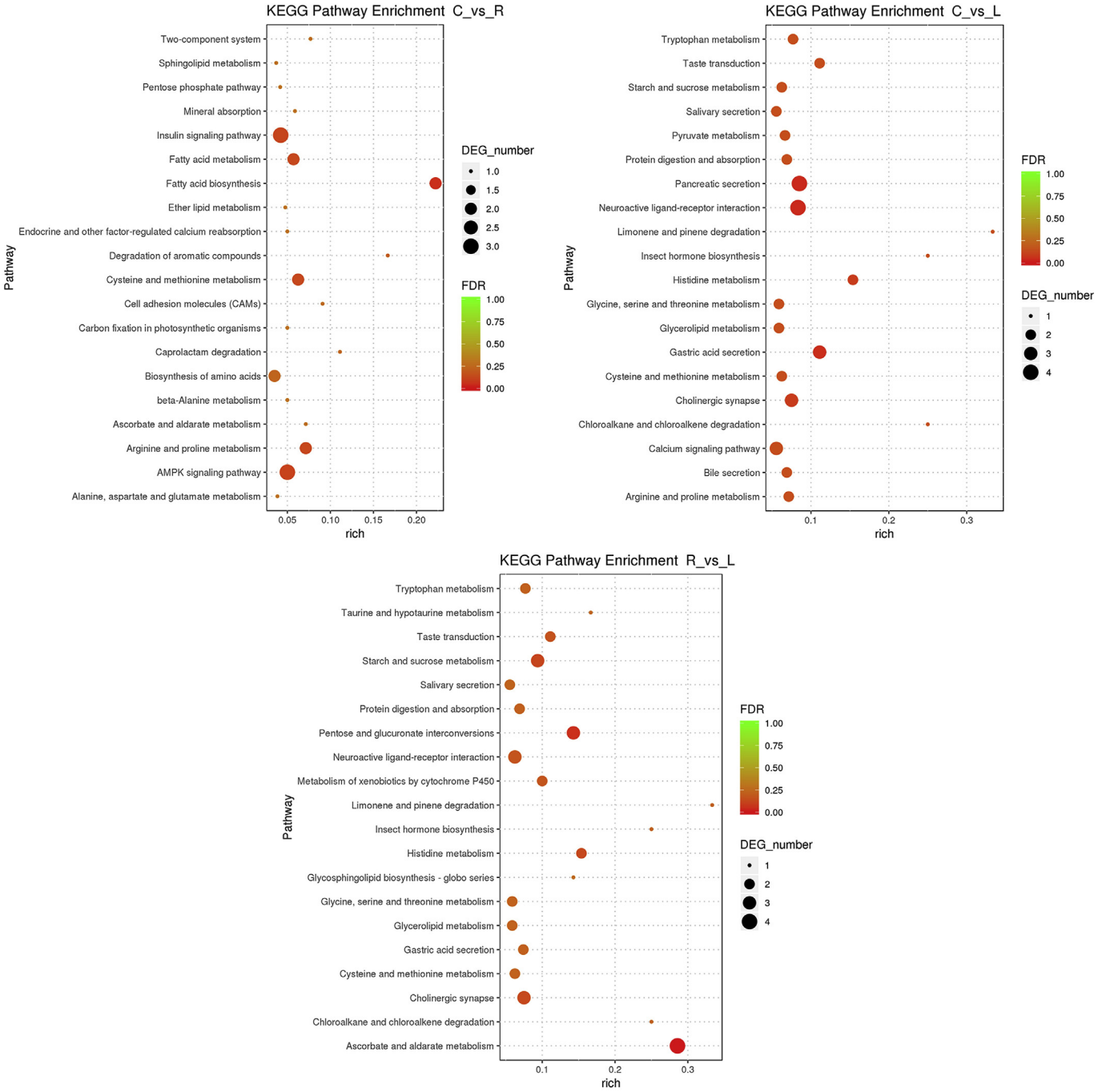

Fig. 9. KEGG enrichment scatterplots of the differentially-expressed genes (DGEs). The vertical axis represents the pathway name, the horizontal axis represents the corresponding enrichment factor. The q-value corresponds to color shades: a redder color represents a smaller q-value. The size of the point indicates the number of DEGs in each pathway. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

component of plasma membrane), MF (transmembrane transporter activity, substrate-specific transmembrane transporter activity, ion transmembrane transporter activity, ion channel activity, substrate-specific channel activity, monovalent inorganic cation transmembrane transporter activity).

To understand the pathways affected by HC-2 and LiCl-treated HC-2 fed in the diet, DEGs ($\log_2$ |fold change| > 2, q-value < 0.05) were compared against the KEGG database for pathway enrichment (Fig. 9). The DEGs between R group and the control group mainly enriched in AMPK signaling pathway, insulin signaling pathway, fatty acid biosynthesis, biosynthesis of amino acids and arginine and proline metabolism. The DEGs between L group and the control group mainly enriched in pancreatic secretion, neuroactive ligand-receptor interaction, gastric acid secretion, cholinergic synapse and calcium signaling pathway. The DEGs between R group and L group mainly enriched in ascorbate and aldarate metabolism, pentose and glucuronate

Table 2
Candidate genes involved in the comparison of R samples with the control samples.

Category or Gene ID	Homologues function	Species	FC[a]
Immune response			
TRINITY_DN75754_c0_g1	Protein spaetzle 3	*Drosophila melanogaster*	4.57
TRINITY_DN79309_c2_g1	Proclotting enzyme	*Tachypleus tridentatus*	2.30
TRINITY_DN70500_c1_g4	Uncharacterized peptidase C1-like protein F26E4.3	*Caenorhabditis elegans*	2.92
TRINITY_DN81707_c0_g1	NACHT, LRR and PYD domains-containing protein 10	*Homo sapiens*	6.20
TRINITY_DN58900_c0_g1	Macrophage mannose receptor 1	*Mus musculus*	6.59
TRINITY_DN79438_c2_g2	Acid sphingomyelinase-like phosphodiesterase 3b	*Mus musculus*	2.26
TRINITY_DN64655_c0_g1	C-type lectin	*Fenneropenaeus merguiensis*	7.77
TRINITY_DN18542_c0_g1	toll-like receptor	*Portunus trituberculatus*	5.50
TRINITY_DN73731_c2_g1	C-type lectin domain family 4 member E-like isoform X2	*Haplochromis burtoni*	4.53
TRINITY_DN70958_c4_g1	C-type lectin domain-containing protein	*Fenneropenaeus chinensis*	2.06
TRINITY_DN59779_c0_g1	trypsin	*Marsupenaeus japonicus*	10.58
TRINITY_DN62123_c0_g1	invertebrate-type lysozyme protein	*Marsupenaeus japonicus*	8.65
TRINITY_DN69447_c5_g2	Peroxidasin	*Drosophila melanogaster*	3.48
TRINITY_DN83845_c3_g9	integrin alpha 5	*Fenneropenaeus chinensis*	2.76
TRINITY_DN64808_c0_g1	Ubiquitin-activating enzyme E1 1	*Schizosaccharomyces pombe*	3.77
TRINITY_DN81259_c2_g3	integrin beta subunit	*Litopenaeus vannamei*	2.01
TRINITY_DN75992_c4_g2	Heat shock protein 67B2, partial	*Stegodyphus mimosarum*	2.05
TRINITY_DN77853_c1_g4	cathepsin C	*Fenneropenaeus chinensis*	0.33
TRINITY_DN73455_c2_g1	crustin type I	*Macrobrachium rosenbergii*	8.49
TRINITY_DN65636_c0_g1	Anti-lipopolysaccharide factor	*Portunus trituberculatus*	2.42
TRINITY_DN76935_c7_g5	peritrophin	Litopenaeus vannamei	2.52
MAPK signalling pathway			
TRINITY_DN43120_c0_g1	serine proteinase stubble	*Diachasma alloeum*	Inf
TRINITY_DN66579_c0_g1	serine proteinase	*Fenneropenaeus chinensis*	2.06
TRINITY_DN58649_c0_g1	putative serine proteinase inhibitor	*Pacifastacus leniusculus*	13.76
TRINITY_DN78155_c3_g1	putative serine proteinase inhibitor	*Pacifastacus leniusculus*	5.35
TRINITY_DN75725_c5_g1	serine proteinase	*Litopenaeus vannamei*	3.01
TRINITY_DN71574_c0_g1	Kazal-type serine proteinase inhibitor 2	*Procambarus clarkii*	3.95
TRINITY_DN71967_c2_g1	serine proteinase inhibitor 8	*Penaeus monodon*	2.93
TRINITY_DN43120_c0_g1	serine proteinase stubble	*Diachasma alloeum*	Inf
Vascular endothelial growth factor			
TRINITY_DN69210_c0_g1	vascular endothelial growth factor 2	*Litopenaeus vannamei*	2.47
TRINITY_DN69418_c2_g1	vascular endothelial growth factor 2	*Litopenaeus vannamei*	2.89
TRINITY_DN81118_c2_g2	vascular endothelial growth factor receptor precursor	*Litopenaeus vannamei*	2.48
TRINITY_DN82014_c3_g4	vascular endothelial growth factor receptor precursor	*Litopenaeus vannamei*	2.36
TRINITY_DN84416_c3_g3	vascular endothelial growth factor receptor precursor	*Litopenaeus vannamei*	2.52
TRINITY_DN71657_c2_g3	vascular endothelial growth factor receptor 1 isoform X5	*Tribolium castaneum*	2.38
TRINITY_DN71657_c2_g2	vascular endothelial growth factor receptor 1-like isoform X3	*Cynoglossus semilaevis*	3.13
cell surface receptor signaling pathway			
TRINITY_DN75754_c0_g1	Protein spaetzle 3	*Drosophila melanogaster*	4.57
TRINITY_DN83956_c1_g2	Adhesion G-protein coupled receptor G2	*Mus musculus*	3.35
TRINITY_DN49258_c0_g1	Neuronal acetylcholine receptor subunit alpha-10	*Gallus gallus*	0.18
TRINITY_DN77224_c3_g3	Poly [ADP-ribose] polymerase 2	*Homo sapiens*	2.05
TRINITY_DN71657_c2_g2	Vascular endothelial growth factor receptor 1	*Gallus gallus*	3.13
TRINITY_DN81259_c2_g3	Integrin beta-PS	*Drosophila melanogaster*	2.01
TRINITY_DN75629_c1_g1	Protein shifted OS	*Drosophila melanogaster*	3.38
TRINITY_DN82145_c1_g1	Glutamate receptor ionotropic, kainate 2	*Mus musculus*	2.88
TRINITY_DN70389_c0_g2	Enhancer of split mbeta protein	*Drosophila melanogaster*	2.00
TRINITY_DN80062_c0_g1	Probable G-protein coupled receptor No18	*Amphibalanus amphitrite*	2.69
TRINITY_DN70500_c1_g4	Uncharacterized peptidase C1-like protein F26E4.3	*Caenorhabditis elegans*	2.92
TRINITY_DN127556_c0_g1	Xenotropic and polytropic retrovirus receptor 1	*Neovison vison*	8.60
TRINITY_DN81118_c2_g2	vascular endothelial growth factor receptor precursor	*Litopenaeus vannamei*	2.48
TRINITY_DN58900_c0_g1	Macrophage mannose receptor 1	*Mus musculus*	6.59
TRINITY_DN63464_c1_g1	Neuroligin-1	*Caenorhabditis elegans*	3.97
TRINITY_DN76935_c7_g5	peritrophin	Litopenaeus vannamei	2.52
TRINITY_DN78657_c1_g6	Peroxisome proliferator-activated receptor delta	*Daphnia magna*	5.48
TRINITY_DN77702_c0_g2	trace amine-associated receptor 7d-like	*Takifugu rubripes*	3.37
TRINITY_DN84416_c3_g3	vascular endothelial growth factor receptor precursor	*Litopenaeus vannamei*	2.52
TRINITY_DN83825_c2_g1	Low-density lipoprotein receptor	*Oryctolagus cuniculus*	2.42
Channel			
TRINITY_DN67186_c0_g2	voltage gated sodium channel, partial	*Astacus leptodactylus*	3.45
TRINITY_DN83252_c2_g1	calcium-activated chloride channel regulator 4-like	*Saccoglossus kowalevskii*	2.95
TRINITY_DN77476_c0_g1	Calcium-activated chloride channel regulator 2	*Daphnia magna*	2.69
Cell adhesion			
TRINITY_DN70500_c1_g4	Uncharacterized peptidase C1-like protein F26E4.3	*Caenorhabditis elegans*	2.92
TRINITY_DN81259_c2_g3	integrin beta subunit	*Litopenaeus vannamei*	2.01
TRINITY_DN82651_c2_g1			
TRINITY_DN63464_c1_g1	Neuroligin-1	*Caenorhabditis elegans*	3.97
Response to stress			

(*continued on next page*)

Table 2 (*continued*)

Category or Gene ID	Homologues function	Species	FC[a]
TRINITY_DN77224_c3_g3	Poly [ADP-ribose] polymerase 2	*Homo sapiens*	2.05
TRINITY_DN75754_c0_g1	Protein spaetzle 3	*Drosophila melanogaster*	4.57
TRINITY_DN79309_c2_g1	Proclotting enzyme	Tachypleus tridentatus	2.30
TRINITY_DN73399_c0_g1	Chorion peroxidase	*Drosophila melanogaster*	4.74
TRINITY_DN58900_c0_g1	Macrophage mannose receptor 1	*Mus musculus*	6.59
TRINITY_DN81707_c0_g1	NACHT, LRR and PYD domains-containing protein 10	*Homo sapiens*	6.20
TRINITY_DN72921_c1_g1	Multidrug resistance-associated protein 1	*Rattus norvegicus*	2.44
TRINITY_DN82145_c1_g1	Glutamate receptor ionotropic, kainate 2	*Mus musculus*	2.87
TRINITY_DN69447_c5_g2	Peroxidasin	*Drosophila melanogaster*	3.48
TRINITY_DN77476_c0_g1	Calcium-activated chloride channel regulator 1	*Homo sapiens*	2.69
TRINITY_DN65636_c0_g1	Anti-lipopolysaccharide factor	Portunus trituberculatus	2.42
TRINITY_DN79438_c2_g2	Acid sphingomyelinase-like phosphodiesterase 3b	*Mus musculus*	2.26
Response to stimulus			
TRINITY_DN80062_c0_g1	Probable G-protein coupled receptor No18	*Amphibalanus amphitrite*	2.69
TRINITY_DN65636_c0_g1	Anti-lipopolysaccharide factor	*Portunus trituberculatus*	2.42
TRINITY_DN70958_c4_g1	Tetranectin	*Homo sapiens*	2.06
TRINITY_DN70500_c1_g4	Uncharacterized peptidase C1-like protein F26E4.3	*Caenorhabditis elegans*	2.92
TRINITY_DN81707_c0_g1	NACHT, LRR and PYD domains-containing protein 10	*Homo sapiens*	6.20
TRINITY_DN80300_c2_g1	Probable 3′,5′-cyclic phosphodiesterase pde-5	*Caenorhabditis elegans*	2.20
TRINITY_DN74231_c6_g1	hypothetical protein OCBIM_22012146 mg, partial	*Octopus bimaculoides*	0.49
TRINITY_DN49258_c0_g1	Neuronal acetylcholine receptor subunit alpha-10	*Gallus gallus*	0.18
TRINITY_DN70835_c4_g1	Ribosomal protein S6 kinase beta-1	*Mus musculus*	0.48
TRINITY_DN79309_c2_g1	Proclotting enzyme	*Tachypleus tridentatus*	2.30
TRINITY_DN81259_c2_g3	Integrin beta-PS	*Drosophila melanogaster*	2.01
TRINITY_DN77951_c0_g1	Chorion peroxidase	*Drosophila melanogaster*	3.98
TRINITY_DN58900_c0_g1	Macrophage mannose receptor 1	*Mus musculus*	6.59
TRINITY_DN79438_c2_g2	Acid sphingomyelinase-like phosphodiesterase 3b	*Mus musculus*	2.26
TRINITY_DN70389_c0_g2	Enhancer of split mbeta protein	*Drosophila melanogaster*	2.00
TRINITY_DN75754_c0_g1	Protein spaetzle 3	*Drosophila melanogaster*	4.57
TRINITY_DN83956_c1_g2	Adhesion G-protein coupled receptor G2	*Mus musculus*	3.35
TRINITY_DN69447_c5_g2	Peroxidasin	*Drosophila melanogaster*	3.48
TRINITY_DN77476_c0_g1	Calcium-activated chloride channel regulator 1	*Homo sapiens*	2.69
TRINITY_DN82487_c3_g1	SH3 domain-binding protein 5 homolog	*Drosophila melanogaster*	0.43
TRINITY_DN81952_c2_g3	Probable G-protein coupled receptor Mth-like 1	*Drosophila melanogaster*	2.42
TRINITY_DN77224_c3_g3	Poly [ADP-ribose] polymerase 2	*Homo sapiens*	2.05
TRINITY_DN80450_c1_g1	ADP-ribosylation factor 4	*Xenopus laevis*	2.15
TRINITY_DN71657_c2_g2	Vascular endothelial growth factor receptor 1	*Gallus gallus*	3.13
TRINITY_DN72921_c1_g1	Multidrug resistance-associated protein 1	*Rattus norvegicus*	2.44
TRINITY_DN73399_c0_g1	Chorion peroxidase	*Drosophila melanogaster*	4.74
TRINITY_DN82145_c1_g1	Glutamate receptor ionotropic, kainate 2	*Mus musculus*	2.88
TRINITY_DN75629_c1_g1	Protein shifted OS	*Drosophila melanogaster*	3.38
TRINITY_DN70513_c0_g3	hypothetical protein D910_04416, partial	*Dendroctonus ponderosae*	0.09
oxidoreductase activity			
TRINITY_DN67654_c6_g1	Cytochrome P450 2L1	Panulirus argus	3.52
TRINITY_DN67654_c3_g1	Cytochrome P450 2L1	Panulirus argus	0.47
TRINITY_DN84085_c5_g4	probable cytochrome P450 6a13	Cerapachys biroi	0.10
TRINITY_DN84085_c5_g3	cytochrome P450 CYP6BQ22	Dastarcus helophoroides	0.18
TRINITY_DN61785_c0_g1	Cytochrome P450 6a2-like Protein	*Tribolium castaneum*	0.18
TRINITY_DN70505_c4_g3	Cytochrome P450 3A29	*Crassostrea gigas*	2.49
TRINITY_DN70084_c0_g4	cytochrome b	Fenneropenaeus chinensis	0.23
endopeptidase activity			
TRINITY_DN83183_c3_g2	Neprilysin-3	*Drosophila melanogaster*	2.88
TRINITY_DN59779_c0_g1	Cationic trypsin	*Bos taurus*	10.58
TRINITY_DN84445_c5_g1	Transposon Ty3-I Gag-Pol polyprotein	*Saccharomyces cerevisiae*	2.25
TRINITY_DN83512_c0_g1	Transposon Tf2-9 polyprotein	*Schizosaccharomyces pombe*	4.30
TRINITY_DN77476_c0_g1	Calcium-activated chloride channel regulator 1	*Homo sapiens*	2.69
TRINITY_DN43120_c0_g1	Serine proteinase stubble	*Drosophila melanogaster*	Inf
TRINITY_DN77853_c1_g4	Dipeptidyl peptidase 1	*Pongo abelii*	0.33
TRINITY_DN66579_c0_g1	Melanization protease 1	*Drosophila melanogaster*	2.06
TRINITY_DN79309_c2_g1	Serine protease	*Fenneropenaeus chinensis*	2.30
TRINITY_DN75725_c5_g1	Proclotting enzyme	*Tachypleus tridentatus*	3.01
TRINITY_DN50890_c0_g1	Transposon Ty3-G Gag-Pol polyprotein	*Saccharomyces cerevisiae*	6.00
TRINITY_DN73664_c0_g1	Serine proteinase stubble	*Drosophila melanogaster*	2.21
hydrolase activity, acting on ester bonds			
TRINITY_DN84445_c5_g1	Transposon Ty3-I Gag-Pol polyprotein	*Saccharomyces cerevisiae*	2.25
TRINITY_DN83512_c0_g1	Transposon Tf2-9 polyprotein	*Schizosaccharomyces pombe*	4.30
TRINITY_DN80300_c2_g1	Probable 3′,5′-cyclic phosphodiesterase pde-5	*Caenorhabditis elegans*	2.20
TRINITY_DN79438_c2_g3	Acid sphingomyelinase-like phosphodiesterase 3a	*Bos taurus*	5.34
TRINITY_DN77930_c5_g1	Regucalcin	*Sus scrofa*	3.13
TRINITY_DN68586_c2_g1	Dual specificity protein phosphatase 14	*Homo sapiens*	4.41
TRINITY_DN80658_c1_g5	Venom carboxylesterase-6	*Apis mellifera*	8.20

(*continued on next page*)

Table 2 (*continued*)

Category or Gene ID	Homologues function	Species	FC[a]
TRINITY_DN83483_c0_g1	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase TPTE2	*Macaca fascicularis*	2.03
TRINITY_DN75091_c0_g3	Pancreatic triacylglycerol lipase	*Mus musculus*	2.93
TRINITY_DN50890_c0_g1	Transposon Ty3-G Gag-Pol polyprotein	*Saccharomyces cerevisiae*	6.00
TRINITY_DN83107_c0_g1	Fatty acid synthase	*Homo sapiens*	2.17
TRINITY_DN83107_c0_g2	Fatty acid synthase	*Rattus norvegicus*	2.27
TRINITY_DN79438_c2_g2	Acid sphingomyelinase-like phosphodiesterase 3b	*Mus musculus*	2.26
molecular transducer activity			
TRINITY_DN80062_c0_g1	Probable G-protein coupled receptor No18	*Amphibalanus amphitrite*	2.69
TRINITY_DN83956_c1_g2	Adhesion G-protein coupled receptor G2	*Mus musculus*	3.35
TRINITY_DN70500_c1_g4	Uncharacterized peptidase C1-like protein F26E4.3	*Caenorhabditis elegans*	2.92
TRINITY_DN127556_c0_g1	Xenotropic and polytropic retrovirus receptor 1	*Neovison vison*	8.60
TRINITY_DN49258_c0_g1	Neuronal acetylcholine receptor subunit alpha-10	*Gallus gallus*	0.18
TRINITY_DN81118_c2_g2	vascular endothelial growth factor receptor precursor	*Litopenaeus vannamei*	2.48
TRINITY_DN71657_c2_g2	Vascular endothelial growth factor receptor 1	*Gallus gallus*	3.13
TRINITY_DN82145_c1_g1	Glutamate receptor ionotropic, kainate 2	*Mus musculus*	2.88
TRINITY_DN58900_c0_g1	Macrophage mannose receptor 1	*Mus musculus*	6.59
TRINITY_DN63464_c1_g1	Neuroligin-1	*Caenorhabditis elegans*	3.97
peptidase activity, acting on L-amino acid peptides			
TRINITY_DN83183_c3_g2	Neprilysin-3	*Drosophila melanogaster*	2.88
TRINITY_DN59779_c0_g1	Cationic trypsin	*Bos taurus*	10.58
TRINITY_DN84445_c5_g1	Transposon Ty3-I Gag-Pol polyprotein	*Saccharomyces cerevisiae*	2.25
TRINITY_DN83512_c0_g1	Transposon Tf2-9 polyprotein	*Schizosaccharomyces pombe*	4.30
TRINITY_DN77476_c0_g1	Calcium-activated chloride channel regulator 1	*Homo sapiens*	2.69
TRINITY_DN70500_c1_g4	Uncharacterized peptidase C1-like protein F26E4.3	*Caenorhabditis elegans*	2.92
TRINITY_DN43120_c0_g1	Serine proteinase stubble	*Drosophila melanogaster*	Inf
TRINITY_DN77853_c1_g4	Dipeptidyl peptidase 1	*Pongo abelii*	0.33
TRINITY_DN66579_c0_g1	Melanization protease 1	*Drosophila melanogaster*	2.06
TRINITY_DN83252_c2_g1	Calcium-activated chloride channel regulator 3A-1	*Mus musculus*	2.95
TRINITY_DN79309_c2_g1	Proclotting enzyme	*Tachypleus tridentatus*	2.30
TRINITY_DN75725_c5_g1	Proclotting enzyme	*Tachypleus tridentatus*	3.01
TRINITY_DN50890_c0_g1	Transposon Ty3-G Gag-Pol polyprotein	*Saccharomyces cerevisiae*	6.00
TRINITY_DN73664_c0_g1	Serine proteinase stubble	*Drosophila melanogaster*	2.21
TRINITY_DN78453_c2_g1	Caspase-2	*Mus musculus*	2.23

[a] Fold changes (Log_2 ratio) in gene expression. Inf indicate gene was expressed in the challenge group but not in the control group.

interconversions, starch and sucrose metabolism, taste transduction and metabolism of xenobiotics by cytochrome P450.

3.5. Genes potentially involved in shrimp immune response, cell adhesion and metabolic

According to the GO and KEGG analysis, several of the genes that were differentially expressed in midguts of shrimp among the three treatment groups are involved in immune system (Immune response, MAPK signalling pathway, vascular endothelial growth factor, response to stress, response to stimulus, oxidoreductase activity, endopeptidase activity, hydrolase activity, acting on ester bonds, heat shock protein), cell-cell adhesion (vascular endothelial growth factor, cell surface receptor signaling pathway, channel, cell adhesion, molecular transducer activity, active transmembrane transporter activity, receptor activity) and metabolic (hydrolase activity, acting on ester bonds, peptidase activity, acting on L-amino acid peptides) (Tables 2–4).

3.6. Validation of transcriptome data by qPCR

In the present study, 8 DEGs involved in the immune system were randomly selected to validate their expression patterns in the transcriptome results by qPCR. The qPCR results were consistent with the transcriptome data (Fig. 10), which further confirmed the reliability of RNA-seq and the accuracy of Trinity assembly.

4. Discussion

Probiotics (i.e., health-promoting bacteria) are live non-pathogenic microorganisms (bacteria or yeast) that are able to colonize in the intestines in sufficient numbers to promote health benefits to the host [40,41]. Lactobacilli, which represent an important part of the natural gut microbiome in both humans and animals have been intensively explored as probiotics [42]. Recent years, increasing attention has been paid to the utilization of Lactic acid bacteria as probiotic to regulate the intestinal immune response, digestion and metabolism, microbial communities to defend the shrimp gastro-intestinal disease. However, the genetic basis of *Lactobacillus* plays a physiological role in intestine based on the surface molecules has been poorly defined. Therefore, in order to reveal the mechanism of probiotic function in shrimp gut induced by the Lactobacilli, we conducted experiments to explore the roles surface proteins of *Lactobacilli* involved in the probiotic-process at molecular levels.

Oxidation stress response is an important defense mechanism of crustaceans against invading microorganisms that produce a large amount of reactive oxygen species (ROS) [43]. However, overproduction of ROS and residual ROS also cause serious damage to cells and tissues. To protect cells against oxidative stress and prevent or repair oxidative damage, cells have developed a set of antioxidant defense system, including many antioxidant enzymes, such as SOD and CAT [44]. Alkaline phosphatase (ACP) is an important immune active factor in humoral immune defense mechanism of *L. vannamei*. Several studies have reported that probiotics could induce the immune enzyme levels to protect the shrimp from pathogens, for example, *L. vannamei* fed with *Enterobacter hormaechei* (E3) and *Lactobacillus* (L3) probiotics significantly elevated the immune enzyme (SOD, PPO, ACP, POD, CAT, LZM) activity and survival rate after WSSV infection [45]. In this study, the immune enzyme activities of SOD, ACP and CAT were increased significantly higher in *L. vannamei* fed with normal HC-2 than the control or LiCl-treated HC-2 group during the feeding and challenge experiment, indicating that surface proteins were vital factor for HC-2 to improve shrimp immune response by strengthen the immune-related

Table 3
Candidate genes involved in the comparison of L samples with the control samples.

Category or Gene ID	Homologues function	Species	FC[a]
oxidoreductase activity			
TRINITY_DN83298_c1_g2	Cytochrome P450 2L1	*Panulirus argus*	0.49
TRINITY_DN69156_c1_g1	DBH-like monooxygenase protein 1	*Mus musculus*	Inf
TRINITY_DN82674_c3_g5	Cytochrome P450 9b1	*Drosophila melanogaster*	0.48
TRINITY_DN83389_c3_g3	Xanthine dehydrogenase/oxidase	*Gallus gallus*	0.44
TRINITY_DN72451_c0_g1	Cytochrome P450 2L1	*Panulirus argus*	0.43
TRINITY_DN84213_c1_g3	Cytochrome P450 2L1	*Panulirus argus*	0.47
TRINITY_DN83353_c0_g1	Dimethylglycine dehydrogenase, mitochondrial	*Mus musculus*	0.19
TRINITY_DN82950_c0_g5	oxidase 5	*Homo sapiens*	2.47
TRINITY_DN84213_c1_g2	Cytochrome P450 2L1	*Panulirus argus*	0.43
TRINITY_DN82401_c1_g1	Delta-1-pyrroline-5-carboxylate synthase	*Homo sapiens*	0.48
TRINITY_DN80398_c0_g1	NADPH–cytochrome P450 reductase	*Drosophila melanogaster*	0.45
TRINITY_DN83839_c2_g2	Cytochrome P450 CYP2	*Eriocheir sinensis*	0.49
TRINITY_DN81502_c1_g1	Cytochrome P450 9e2	*Blattella germanica*	0.44
TRINITY_DN64396_c0_g1	Cytochrome P450 18a1	*Drosophila melanogaster*	6.74
TRINITY_DN82401_c1_g2	Delta-1-pyrroline-5-carboxylate synthase	*Mus musculus*	0.44
TRINITY_DN79943_c2_g3	Glyceraldehyde-3-phosphate dehydrogenase	*Panulirus versicolor*	0.17
TRINITY_DN82666_c1_g1	Malate dehydrogenase	*Pyrococcus horikoshii*	0.22
TRINITY_DN83298_c1_g1	Cytochrome P450 CYP2	*Eriocheir sinensis*	0.50
TRINITY_DN70463_c5_g3	Aldehyde dehydrogenase, dimeric NADP-preferring	*Homo sapiens*	0.36
TRINITY_DN81638_c1_g5	Cytochrome P450 2L1	*Panulirus argus*	0.49
TRINITY_DN72921_c1_g1	Multidrug resistance-associated protein 1	*Rattus norvegicus*	2.90
TRINITY_DN69764_c4_g1	Fatty acid hydroxylase domain-containing protein 2	*Homo sapiens*	0.43
TRINITY_DN65738_c0_g1	Prostaglandin reductase 1	*Bos taurus*	0.50
TRINITY_DN82674_c1_g1	Cytochrome P450 9e2	*Blattella germanica*	0.37
active transmembrane transporter activity			
TRINITY_DN76107_c11_g1	Sodium- and chloride-dependent glycine transporter 1	*Xenopus laevis*	0.45
TRINITY_DN84422_c9_g4	Multidrug resistance protein 1	*Homo sapiens*	0.40
TRINITY_DN69713_c3_g3	Multidrug resistance protein 1A	*Mus musculus*	0.40
TRINITY_DN81384_c2_g5	Sodium-dependent nutrient amino acid transporter 1	*Drosophila ananassae*	2.08
TRINITY_DN67016_c9_g1	Multidrug resistance protein homolog 49	*Drosophila melanogaster*	0.14
TRINITY_DN84422_c9_g2	ATP-binding cassette sub-family B member 5	*Mus musculus*	0.20
TRINITY_DN72921_c1_g1	Multidrug resistance-associated protein 1	*Rattus norvegicus*	2.90
TRINITY_DN83448_c2_g1	Sodium/calcium exchanger 1	*Rattus norvegicus*	0.41
TRINITY_DN79697_c3_g1	Sodium/calcium exchanger 1	*Mus musculus*	0.45
TRINITY_DN84422_c9_g1	Multidrug resistance protein 1	*Cricetulus griseus*	0.40
TRINITY_DN81384_c2_g4	Sodium-dependent nutrient amino acid transporter 1	*Drosophila mojavensis*	2.37
TRINITY_DN64717_c1_g1	Sodium-dependent nutrient amino acid transporter 1	*Drosophila grimshawi*	2.35
Immune response			
TRINITY_DN63138_c0_g1	C-type lectin	*Portunus trituberculatus*	0.23
TRINITY_DN69571_c3_g2	lectin	*Procambarus clarkii*	0.20
TRINITY_DN72692_c4_g1	C-type lectin	*Portunus trituberculatus*	0.35
TRINITY_DN59779_c0_g1	trypsin	*Marsupenaeus japonicus*	8.48
TRINITY_DN68874_c0_g1	Protein spaetzle 5	*Drosophila melanogaster*	2.58
TRINITY_DN77853_c1_g1	Dipeptidyl peptidase 1	*Mus musculus*	0.19
TRINITY_DN77853_c1_g4	Cathepsin C	*Fenneropenaeus chinensis*	0.36
TRINITY_DN70207_c4_g2	peritrophin-44-like protein	*Eriocheir sinensis*	0.07
Heat shock protein			
TRINITY_DN65743_c10_g1	heat shock protein 90-2	*Portunus trituberculatus*	0.34
TRINITY_DN71579_c1_g3	heat shock protein 70	*Portunus trituberculatus*	0.42
TRINITY_DN72754_c1_g2	heat shock cognate 70, partial	*Macrobrachium amazonicum*	0.30
TRINITY_DN72754_c2_g3	heat shock protein 70	*Litopenaeus vannamei*	0.37
TRINITY_DN76667_c1_g1	PREDICTED: protein lethal(2)essential for life-like	*Copidosoma floridanum*	0.39
TRINITY_DN83667_c5_g4	HSP90	*Scylla paramamosain*	0.35
TRINITY_DN85852_c0_g1	Probable heat shock factor protein homolog	*Enterocytozoon bieneusi*	0.00
Channel			
TRINITY_DN73670_c1_g1	voltage-dependent calcium channel type A subunit alpha-1 isoform X7	*Cephus cinctus*	0.45
TRINITY_DN75162_c3_g1	two pore potassium channel protein sup-9-like	*Limulus polyphemus*	0.25
TRINITY_DN77251_c1_g1	TWiK family of potassium channels protein 7	*Plutella xylostella*	0.44
TRINITY_DN79505_c2_g2	sodium channel protein 60E isoform X5	*Tribolium castaneum*	0.45
TRINITY_DN81740_c3_g1	potassium voltage-gated channel subfamily KQT member 1-like isoform X5	*Apis mellifera*	0.49
TRINITY_DN82570_c3_g3	Glutamate-gated chloride channel, partial	*Stegodyphus mimosarum*	0.43
TRINITY_DN82570_c3_g2	glutamate gated chloride channel	*Bombyx mori*	0.47
Receptor activity			
TRINITY_DN66889_c7_g1	Probable nuclear hormone receptor HR3	*Drosophila melanogaster*	3.60
TRINITY_DN74079_c0_g2	Probable G-protein coupled receptor B0563.6	*Caenorhabditis elegans*	4.41
TRINITY_DN78922_c3_g4	Adhesion G protein-coupled receptor L3	*Homo sapiens*	2.05
TRINITY_DN81827_c0_g1	Variant Ionotropic Glutamate Receptor, partial	*Coenobita clypeatus*	4.38
TRINITY_DN83825_c2_g1	Low-density lipoprotein receptor (Fragment)	*Oryctolagus cuniculus*	4.40

(*continued on next page*)

Table 3 (*continued*)

Category or Gene ID	Homologues function	Species	FC[a]
TRINITY_DN68650_c2_g2	Glutamate [NMDA] receptor subunit 1	*Drosophila melanogaster*	0.50
TRINITY_DN70221_c9_g1	G-protein coupled receptor GRL101	*Lymnaea stagnalis*	0.30
TRINITY_DN73657_c3_g9	Muscarinic acetylcholine receptor DM1	*Drosophila melanogaster*	0.35
TRINITY_DN73983_c4_g1	Acetylcholine receptor subunit alpha-like	*Manduca sexta*	0.45
TRINITY_DN75548_c2_g1	Probable glutamate receptor	*Anas platyrhynchos*	0.45
TRINITY_DN77335_c0_g1	Muscarinic acetylcholine receptor DM1	*Drosophila melanogaster*	0.41
TRINITY_DN78844_c3_g1	Receptor-type tyrosine-protein phosphatase beta	*Homo sapiens*	0.23
TRINITY_DN79342_c5_g3	Toll-like receptor 6	*Drosophila melanogaster*	0.45
TRINITY_DN79770_c0_g1	Neuronal acetylcholine receptor subunit alpha-7	*Mus musculus*	0.49
TRINITY_DN80628_c2_g2	G-protein coupled receptor moody	*Drosophila melanogaster*	0.41
TRINITY_DN83173_c2_g5	Glutamate receptor ionotropic, NMDA 2B	*Canis lupus familiaris*	0.37
TRINITY_DN83547_c0_g1	Relaxin receptor 2	*Mus musculus*	0.25
TRINITY_DN83732_c3_g4	Epidermal growth factor receptor	*Apis mellifera*	0.27
TRINITY_DN83732_c3_g3	Epidermal growth factor receptor	*Drosophila melanogaster*	0.31

[a] Fold changes (Log_2 ratio) in gene expression. Inf indicate gene was expressed in the challenge group but not in the control group.

enzymes.

Trypsin, lipase and α-amylase are the vital digestive enzymes involved in the assimilation of nutrition in the gut of shrimp [46]. The digestive enzyme levels was usually used for evaluating the food conversion efficiency and growth performance in shrimp culture, many studies have reported that diet with probiotics increased the trypsin, lipase and α-amylase [47–49]. In this study, we found that normal HC-2 and LiCl-treated both contributed to the increasing of these three digestive enzymes activities during the feeding, but the enzyme levels were significantly higher in R group than in L group. This indicated that surface proteins were important factor for the probiotics to regulate the digestion in gut of shrimp.

Probiotic treatment could enhance the shrimp immune responses as shown in some previous studies [11,50,51]. By analyzing transcriptome data, we found that feeding with the normal HC-2 induced the immune-related genes up-regulation, but these genes were down-regulation in LiCl-treated HC-2 group, which suggesting surface proteins play vital roles in mediation HC-2 improve the shrimp intestinal immune response.

The first step of lactobacillus adhesion process is non-specific, which is determined by the specificity of bacterial structure. On the basis of nonspecific conjugation, the specific ligands of the bacteria further bind to the corresponding receptors of the host cells [52]. Studies have shown that the interaction between lactobacillus and the host is mainly related to the hydrophobicity and self-agglutination of the bacterial surface, lipoteichoicacid (LTA), exopolysaccharides (EPS) and related to cell surface proteins [25,53]. The surface proteins of lactobacillus including s-layer proteins, sortase-dependent proteins (SDPs), mucosal binding proteins and regulatory surface proteins of extracellular interstitial adhesion were studied showed have important functions such as adhesion, signaling, and interaction with the host immune system or environment. Lactobacillus adhere to host intestinal mucus, intestinal epithelial cells, extracellular stroma by means of its surface proteins, and/or other bacteria lipodesmoic acid to effectively prevent pathogenic bacteria from adhesion to epithelial cells [54–56]. In present study, we found that the genes involved in cell surface receptor signaling pathway were up-regulated in shrimp midgut after fed with the normal HC-2, but the fed with surface proteins shaving bacteria didn't induce these genes up-regulation. Additionally, several studies reported that channel proteins such as voltage gated sodium channel protein, potassium/sodium channel protein, glutamate-gated chloride channel protein, calcium-activated chloride channel protein have mediation of signal transduction, water and ion homeostasis, cell-cell adhesion, cell migration [57–60]. Our results showed that the genes transcript levels of transmembrane transporter activity and channel activity related genes were significantly higher in R group than that in the control group, however, these genes were down-regulated significantly lower in L group than that in the control group. These results indicated that surface proteins were crucial factor for HC-2 adhesion and colonization in the shrimp midgut, and were contributed to activation of a series of molecular signals communication with the surface cell of host.

The improvement in growth can be attained with dietary supplementation with probiotics has previously been attributed to the physiological and biological changes in the intestinal environment as well as morphological changes in the gut epithelium [61,62], such as an improved intestinal microvillus structure and a greater absorptive surface area [63,64]. In the present work, the vascular endothelial growth factor were up-regulated after the shrimp fed with normal HC-2, but the HC-2 with “shaving off” of the surface proteins by LiCl-treatment could not induced vascular endothelial growth factor up-expression, suggesting that dietary supplemented with HC-2 could improve the gut endothelial to enhance the growth performance and immune responses of the shrimp, and the surface proteins played important roles in this process. Peritrophic membrane (PM) is a non-cellular structure surrounding the food bolus in invertebrate's midgut, which participate in modulating its permeability and immobilizating the digestive enzymes, actively protect the gut from pathogen contact, and play an important role in the gut immune system [65]. It has been reported that a thickening of the peritrophic membrane in bees and honeybees from colonies fed with probiotic supplements [66,67]. The peritrophin gene was significantly increased after the shrimp fed with normal HC-2, and this result didn't found in the shrimp midgut fed with LiCl-treated HC-2. This might indicate that surface proteins could mediate the bacteria promote the shrimp peritrophic membrane growth to block the adhesion of pathogen and improve shrimp immune response.

Probiotics were usually used for therapy in clinical study due to its competitive inhibition against the pathogenic bacteria, one reason might explain this issue was that probiotics induced the stress-related genes expression and then increased pathogens eradication [68]. In aquaculture systems, the environmental stress is one of the primary contributing factors to aquatic animal diseases outbreaks and serious mortality. Several reports, however, showed that the stress resistance of animal improved by probiotic treatment, for example, *Paralichthys olivaceus* had greater heat tolerance after being treated with a commercial probiotic supplied in the diet and in the rearing water compared to the control [69]. Administered Sea bream with probiotics of *Lactobacillus fructivorans* and *Lac. plantarum* caused changes in two stress indicators including a significantly lower cortisol level and higher heat shock protein 70 gene expression, and significantly lower cumulative mortality in probiotic-treated sea bream subjected to an acute pH stress (from 8.6 to 6.3) [70]. A better stress tolerance to fresh water, 60 salt water and 300 mg L nitrite-N was also found in *L. vannamei* post larvae after being treated with the probiotic, B. subtilis E20 [71]. In this study, we found that the expression levels of response to stress/stimulus genes were up-regulated significantly higher after the shrimp fed with normal HC-2, but the shrimp fed with LiCl-treated HC-2 didn't induce these

Table 4
Candidate genes involved in the comparison of L samples with R samples.

Category or Gene ID	Homologues function	Species	FC[a]
Immune response			
TRINITY_DN72807_c0_g1	Heat shock protein 22	*Drosophila melanogaster*	0.31
TRINITY_DN76667_c1_g1	Heat shock protein beta-2	*Homo sapiens*	0.41
TRINITY_DN68797_c0_g1	Lysozyme C	*Fenneropenaeus merguiensis*	0.34
TRINITY_DN68797_c4_g1	Lysozyme C	*Fenneropenaeus merguiensis*	0.38
TRINITY_DN70362_c4_g1	ubiquitin-protein ligase RNF31	*Mus musculus*	0.46
TRINITY_DN117927_c0_g1	E3 ubiquitin-protein ligase ZNRF4	*Mus musculus*	0.08
TRINITY_DN120512_c0_g1	E3 ubiquitin-protein ligase MARCH8	*Xenopus tropicalis*	0.06
channel activity			
TRINITY_DN65028_c0_g1	Voltage gated sodium channel, partial	*Astacus leptodactylus*	0.45
TRINITY_DN83370_c2_g1	voltage gated sodium channel, partial	*Astacus leptodactylus*	0.44
TRINITY_DN67186_c0_g1	voltage gated sodium channel, partial	*Astacus leptodactylus*	0.23
TRINITY_DN72021_c0_g1	hyperpolarization-activated cyclic nucleotide-modulated cation channel splice variant ABL-II	*Panulirus interruptus]*	0.24
TRINITY_DN73677_c0_g4	hyperpolarization-activated cyclic nucleotide-modulated cation channel splice variant Bs-I	*Panulirus interruptus]*	0.42
TRINITY_DN67186_c0_g2	Sodium channel protein para	*Drosophila melanogaster*	0.23
TRINITY_DN83751_c3_g1	Potassium voltage-gated channel protein Shaker	*Drosophila melanogaster*	0.46
TRINITY_DN81740_c3_g1	Potassium voltage-gated channel subfamily KQT member 1	*Squalus acanthias*	0.47
TRINITY_DN73677_c0_g2	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4	*Homo sapiens*	0.41
TRINITY_DN76731_c2_g1	Glutamate-gated chloride channel	*Drosophila melanogaster*	0.46
TRINITY_DN82145_c1_g1	Glutamate receptor ionotropic, kainate 2	*Mus musculus*	0.29
TRINITY_DN83252_c2_g1	Calcium-activated chloride channel regulator 3A-1	*Mus musculus*	0.43
TRINITY_DN81763_c4_g4	Bestrophin-3	*Mus musculus*	0.38
transmembrane transporter activity			
TRINITY_DN64717_c1_g1	Sodium-dependent nutrient amino acid transporter 1	*Drosophila grimshawi*	2.69
TRINITY_DN81384_c2_g4	Sodium-dependent nutrient amino acid transporter 1	*Drosophila mojavensis*	2.75
TRINITY_DN81384_c2_g5	Sodium-dependent nutrient amino acid transporter 1	*Drosophila ananassae*	2.25
TRINITY_DN83448_c2_g1	Sodium/calcium exchanger 1	*Rattus norvegicus*	0.50
TRINITY_DN65028_c0_g1	Voltage gated sodium channel, partial	*Astacus leptodactylus*	0.45
TRINITY_DN67186_c0_g1	voltage gated sodium channel, partial	*Astacus leptodactylus*	0.23
TRINITY_DN67186_c0_g2	Sodium channel protein para	*Drosophila melanogaster*	0.23
TRINITY_DN72021_c0_g1	hyperpolarization-activated cyclic nucleotide-modulated cation channel splice variant ABL-II	*Panulirus interruptus]*	0.24
TRINITY_DN73677_c0_g2	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4	*Homo sapiens*	0.41
TRINITY_DN73677_c0_g4	hyperpolarization-activated cyclic nucleotide-modulated cation channel splice variant Bs-I	*Panulirus interruptus]*	0.42
TRINITY_DN81740_c3_g1	Potassium voltage-gated channel subfamily KQT member 1	*Squalus acanthias*	0.47
TRINITY_DN83370_c2_g1	voltage gated sodium channel, partial	*Astacus leptodactylus*	0.44
TRINITY_DN83751_c3_g1	Potassium voltage-gated channel protein Shaker	*Drosophila melanogaster*	0.46
TRINITY_DN76731_c2_g1	Glutamate-gated chloride channel	*Drosophila melanogaster*	0.46
TRINITY_DN78227_c0_g2	Facilitated trehalose transporter Tret1	*Drosophila yakuba*	0.32
TRINITY_DN81384_c2_g2	Sodium-dependent nutrient amino acid transporter 1	*Drosophila pseudoobscura*	2.14
TRINITY_DN81763_c4_g4	Bestrophin-3	*Mus musculus*	0.38
TRINITY_DN82145_c1_g1	Glutamate receptor ionotropic, kainate 2	*Mus musculus*	0.29
TRINITY_DN82213_c2_g5	Sodium-coupled monocarboxylate transporter 1	*Homo sapiens*	2.38
TRINITY_DN83252_c2_g1	Calcium-activated chloride channel regulator 3A-1	*Mus musculus*	0.43
Receptor activity			
TRINITY_DN83956_c1_g2	Adhesion G-protein coupled receptor G2	*Mus musculu*	0.29
TRINITY_DN78873_c2_g2	Probable G-protein coupled receptor Mth-like 1	*Drosophila melanogaster*	0.17
TRINITY_DN66889_c7_g1	hormone receptor HR3	*Thermobia domestica*	4.50
TRINITY_DN82145_c1_g1	Glutamate receptor ionotropic, kainate 2	*Mus musculus*	0.29
TRINITY_DN58900_c0_g1	Macrophage mannose receptor 1	*Mus musculus*	0.21
TRINITY_DN73084_c1_g1	Neuropeptide SIFamide receptor	*Drosophila melanogaster*	0.35
TRINITY_DN64348_c0_g1	beta-2 adrenergic receptor-like	*Xiphophorus maculatus*	0.34
TRINITY_DN68899_c3_g2	PDF receptor-like	*Crassostrea gigas*	0.24
TRINITY_DN71657_c2_g3	Vascular endothelial growth factor receptor 2	*Coturnix japonica*	0.42
TRINITY_DN72566_c2_g1	Glycine receptor subunit alpha-2	*Rattus norvegicus*	0.09
TRINITY_DN73525_c0_g1	Membrane progestin receptor delta	*Mus musculus*	0.48
TRINITY_DN73729_c3_g1	Glutamate receptor ionotropic, delta-1	*Homo sapiens*	0.21
TRINITY_DN75548_c2_g1	Probable glutamate receptor	*Anas platyrhynchos*	0.34
TRINITY_DN76046_c2_g2	G protein-coupled receptor	*Procambarus clarkii*	0.49
TRINITY_DN76170_c0_g1	Acetylcholine receptor subunit alpha-L1	*Schistocerca gregaria*	0.47
TRINITY_DN77335_c0_g1	Muscarinic acetylcholine receptor DM1	*Drosophila melanogaster*	0.41
TRINITY_DN77702_c0_g2	trace amine-associated receptor 7d-like	*Takifugu rubripes*	0.37
TRINITY_DN78657_c1_g6	Vitamin D3 receptor	*Sus scrofa*	0.24
TRINITY_DN79700_c2_g4	vascular endothelial growth factor receptor precursor	*Litopenaeus vannamei*	0.45
TRINITY_DN79770_c0_g1	Neuronal acetylcholine receptor subunit alpha-7	*Mus musculus*	0.40
TRINITY_DN83406_c2_g3	G-protein coupled receptor Mth2	*Drosophila yakuba*	0.47
TRINITY_DN83547_c0_g1	Relaxin receptor 2	*Mus musculus*	0.37
TRINITY_DN84220_c3_g1	nicotinic acetylcholine receptor subunit alpha 10	*Pandalopsis japonica*	0.48
TRINITY_DN84416_c3_g3	Platelet-derived growth factor receptor alpha	*Danio rerio*	0.44
TRINITY_DN121225_c0_g1	ER lumen protein-retaining receptor	*Drosophila melanogaster*	0.03
TRINITY_DN84500_c7_g2	Tyrosine-protein phosphatase non-receptor type 18	*Homo sapiens*	2.75

(*continued on next page*)

Table 4 (*continued*)

Category or Gene ID	Homologues function	Species	FC[a]
oxidoreductase activity			
TRINITY_DN78386_c0_g2	Malate dehydrogenase	*Pyrococcus horikoshii*	0.00
TRINITY_DN83991_c3_g1	UDP-glucose 6-dehydrogenase	*Drosophila melanogaster*	2.03
TRINITY_DN64396_c0_g1	Cytochrome P450 18a1	*Drosophila melanogaster*	6.37
TRINITY_DN82666_c1_g1	Malate dehydrogenase	*Pyrococcus horikoshii*	0.27
TRINITY_DN79943_c2_g3	Glyceraldehyde-3-phosphate dehydrogenase	*Panulirus versicolor*	0.21
TRINITY_DN83098_c0_g1	Peroxidasin	*Drosophila melanogaster*	0.47
TRINITY_DN70463_c5_g3	Aldehyde dehydrogenase, dimeric NADP-preferring	*Homo sapiens*	0.37
TRINITY_DN83353_c0_g1	Dimethylglycine dehydrogenase, mitochondrial	*Mus musculu*	0.17
TRINITY_DN79394_c0_g1	Inositol oxygenase	*Danio rerio*	Inf
TRINITY_DN82950_c0_g5	NADPH oxidase 5	*Homo sapiens*	2.39
TRINITY_DN20444_c0_g1	Probable glycerol-3-phosphate dehydrogenase	*Encephalitozoon cuniculi*	0.15
TRINITY_DN80398_c0_g1	NADPH–cytochrome P450 reductase	*Drosophila melanogaster*	0.45

[a] Fold changes (Log_2 ratio) in gene expression. Inf indicate gene was expressed in the challenge group but not in the control group.

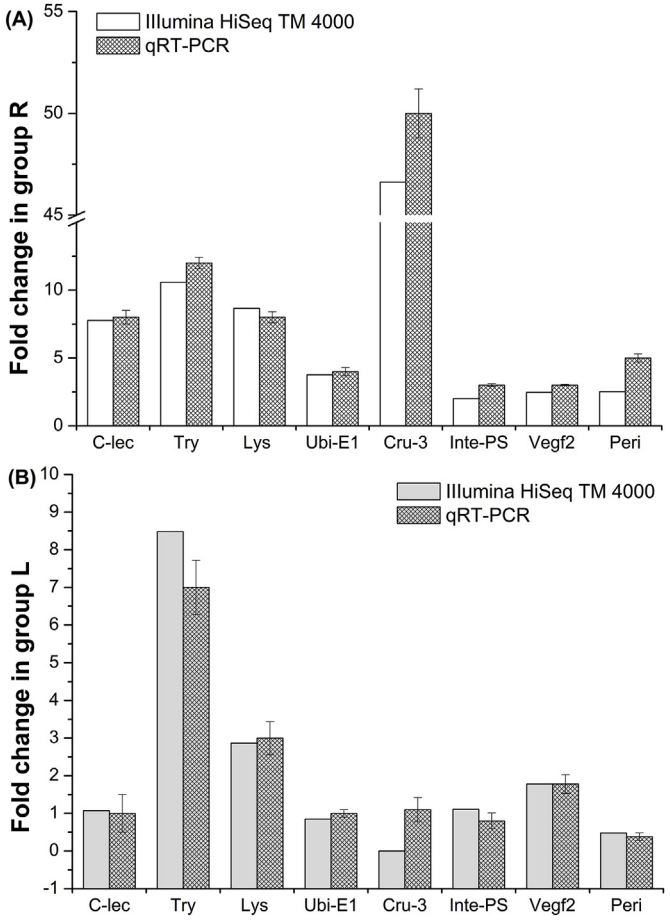

Fig. 10. The expression profiles of selected genes determined by either Illumina sequencing or qRT-PCR. Target gene abbreviations are as follows: C-lec, C-type lectin; Try, trypsin; Lys, invertebrate-type lysozyme protein; Ubi-E1, Ubiquitin-activating enzyme E1 1; Cru-3, crustin 3; Inte-PS, Integrin beta-PS; Vegf2, vascular endothelial growth factor 2; Peri, peritrophin. Data were expressed as the relative fold change (means ± S.E., n = 10). C: shrimp fed a basic commercial diet; R: shrimp fed a basic commercial supplemented with *L. pentosus* HC-2; L: shrimp fed a basic commercial supplemented with LiCl-treated *L. pentosus* HC-2.

genes up-expression, even decreased the heat shock proteins expression. It is thought that the surface proteins were important factors in physiological response of shrimp to the probiotic, *L. pentosus* HC-2, is regulated to adapt to acute environmental stresses.

5. Conclusion

After feeding with normal HC-2 significantly increased activities of antioxidant enzymes and digestion enzymes in midgut of shrimp, and the activities were much higher in the normal HC-2 feeding shrimp than in LiCl-treated HC-2 post challenge. The significantly different genes analysis showed that of *L. vannamei* treated with normal HC-2 induced immune-related, signal transduction, ion homeostasis, cell-cell adhesion, response stress/sitimulus, vascular endothelial growth factor and peritrophin genes up-regulation, but these genes were suppressed in the midgut of shrimp fed with deprived surface proteins bacteria. We believe that the transcriptome information presented in this study will be of great value in understanding the probiotic function from the *L. pentosus* HC-2 to defend the shrimp gastro-intestinal disease by regulation the intestinal immune response, digestion and metabolism, microbial communities.

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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.2019.02.027.

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