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Consumption of *Ulva clathrata* as a dietary supplement stimulates immune and lipid metabolism genes in Pacific white shrimp *Litopenaeus vannamei*

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Abstract Next-generation massive parallel sequencing and bioinformatic techniques were applied to observe the differences in the transcriptome of *Litopenaeus vannamei* juveniles that were fed with a control diet (T1) or the same diet plus a live *Ulva clathrata* supplement (T2). The average size of the libraries was of 271 bp for T1 and 275 bp for T2. A total of 7,706,527 raw reads were obtained with a total of 6,591,856 reads after filtering through Q30, from which 3,855,181 corresponded to T1 and 2,736,675 corresponded to T2. Assembled contigs of each library were annotated using BLASTx from NCBI [obtaining 15,861 (42.77 %) unigenes for T1 and 14,246 (45.06 %) for T2]. In addition, the Kyoto Encyclopedia of Genes and Genomes database (KEGG) as well as the Gene Ontology database (GO) were employed. The differential expression analysis generated a total of 396 transcripts with different expression levels between the two treatments, and these were classified into 4 groups in accordance to their possible function; among others, it identified a percentage of transcripts associated with: immune response (12 %), lipid metabolism (34 %), oxidation-reduction processes (34 %), and stress responses (20 %). The study shows a systematic description of the transcriptome analysis of *L. vannamei* and provides valuable genetic information required for studying the molecular mechanisms operating when shrimp are fed with macroalgae *U. clathrata*.

Keywords *Litopenaeus vannamei* · *Ulva clathrata* · Nutrigenomics · Lipid metabolism · Transcriptome analysis

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Introduction

The Pacific white shrimp, *Litopenaeus vannamei*, is a crustacean species cultured all around the world. It has a considerable commercial value, and its production through farming has surpassed fisheries production, reaching great economic and social importance, as it represents a source of incomes and jobs. For this reason, research on new functional ingredients capable of improving the organism's production has increased; some of these ingredients can play a vital role in the organism's predisposition to fulfill its dietary needs. Several authors have studied different marine algae species as meals, algal extracts, or live algae biomass as supplements in feeds and feeding regimes for shrimp, finding that these ingredients contain bioactive substances such as sulphated polysaccharides associated with immune system stimulation, antiviral, and antioxidant activities (Chotigeat et al. 2004; Rocha de Souza et al. 2007; Wijesekara et al. 2011; Immanuel et al. 2012), polyphenols that are well-known for their antioxidant activity (Bozin et al. 2008; Matanjun et al. 2008; Kumar et al. 2011; Shanab et al. 2011; Simić and Ranković 2012; Trigui et al. 2013; Heffernan et al. 2015) as well as some other lipiphilic compounds such as chlorophyll, carotenoids, and

fatty acids associated with antibacterial and antioxidant activities (Simić and Ranković 2012; Awang et al. 2014).

Ulva clathrata is a macroalga containing vitamins, minerals, carotenoids, polysaccharides, antioxidants and which has a higher protein content compared to other algal species (Cruz-Suárez et al. 2009, 2013). It has been demonstrated that inclusion of *U. clathrata* in the diet of *L. vannamei* improves growth, food conversion ratio, and pigmentation and modifies fatty acid and sterol content in muscle (Cruz-Suárez et al. 2009, 2010, 2013; Peña-Rodríguez et al. 2010, 2011); nevertheless, there are no studies regarding the effects of the dietary inclusion of this alga at a molecular level. The development of new molecular biological techniques, such as massive parallel sequencing methods, have allowed mapping and transcriptome quantification, enabling us to improve our comprehension about the complexity of genomes even in eukaryotic organisms where there is incomplete genome sequencing (Ekblom and Galindo 2011; Guo et al. 2013). With regard to insects, there are studies demonstrating the feasibility of this kind of technology. Such studies have had the objective to understand the organism's response related to nutritional changes and diet quality, in an attempt to improve insect diets (Yocum et al. 2006; Alaux et al. 2011; Zou et al. 2013). In crustaceans, massive parallel sequencing has been employed to identify genes related to immune response, after inoculation with a virus (Li et al. 2013; Zeng et al. 2013; Chen et al. 2013a; Sookruksawong et al. 2013); however, this new tool has not been used in nutritional studies. The present study has as an objective to use next-generation sequencing and bioinformatic techniques in order to obtain information about the *L. vannamei* transcriptome when these shrimp are fed a live *U. clathrata* as a dietary supplement, in order to find an explanation at the molecular level of the benefits of eating seaweed, i.e., genes that change their expression level and metabolic pathways in which these genes are involved. This study also seeks to describe possible molecular mechanisms involved in the improvement of nutritional parameters, for enriching cDNA databases and identifying the genes involved in immune defense and lipid metabolism.

Materials and methods

Experimental diets and animals

The experiment was conducted at the Programa Maricultura facilities (FCB, UANL, Monterrey, Mexico) using specific pathogen-free (SPF) shrimp *L. vannamei* (certificate: DGSA-DSAP-CSAMO-080/2011) obtained from a commercial farm located in Veracruz, Mexico (Langostinos y Camarones de Oriente, S.A. de C.V.). The shrimp were acclimated for 2 weeks prior to the experiment in a recirculating sea water system (35 ± 2 ppt salinity, pH 8.2 ± 0.1, 96.7 ± 1.5 % oxygen) at a temperature of 29 ± 2 °C.

The experiment was conducted using 6 replicated aquaria per treatment. Ten shrimp were allocated to each aquarium (average initial weight 3 ± 0.01 g). The control group (T1) was fed twice a day ad libitum with a control diet (0900 and 1600 h) formulated with 35 % protein and 8.0 % lipids. The experimental group (T2) was fed twice a day (0900 and 1600 h) with the same diet and ad libitum on live *U. clathrata*. Daily, 8 g of living alga was placed in Maya packaging and placed at the bottom of each aquarium at 0900 h and left for 24 h to allow the animals always to have it at their disposal. The next day (before placing the algae again), the remains of algae were weighed and the amount consumed calculated. Both treatment groups were fed over a 20-day period. Feed residue and feces were siphonated out and moults were removed on a daily basis.

Sample collection

Four shrimp per aquarium were sampled after 20 days of feeding. The shrimp that were selected were apparently healthy and in an inter-moult phase. The moulting stage was determined using the technique of Robertson et al. (1987). Subsequently, the hepatopancreas of each shrimp was extracted and individually placed in an Eppendorf tube containing 1 mL RNAlater (Ambion Life Technologies). Approximately 20 mg of each hepatopancreas was sampled in order to perform total extraction of RNA, and the remaining tissue was stored at −80 °C.

RNA extraction and purification

Total RNA from 40 hepatopancreas (20 from each treatment) were extracted using the PureZOL RNA Isolation Reagent (Bio-Rad), in accordance with the manufacturer's instructions. Total RNA quality and integrity were assessed using an Experion RNA StdSens Analysis Kit (Bio-Rad), in accordance with the manufacturer's instructions at Experion Automated Electrophoresis Systems. Eleven samples representing the best quality RNA were selected from each treatment and quantified using the Quant-iT RiboGreen RNA (Invirtogen) assay kit, with the purpose of taking equimolar quantities of each sample and forming a pool for each treatment. The mRNA fraction was purified using RNA Purification Beads in accordance with the TruSeq RNA Sample Preparation v2 Guide (Illumina Part #15026495 Rev. C May 2012) (Fig. 1).

Transcriptome sequencing and bioinformatic analysis

Transcriptome library sequencing was performed using a MiSeq Reagent Kit v2 Illumina next generation sequencer

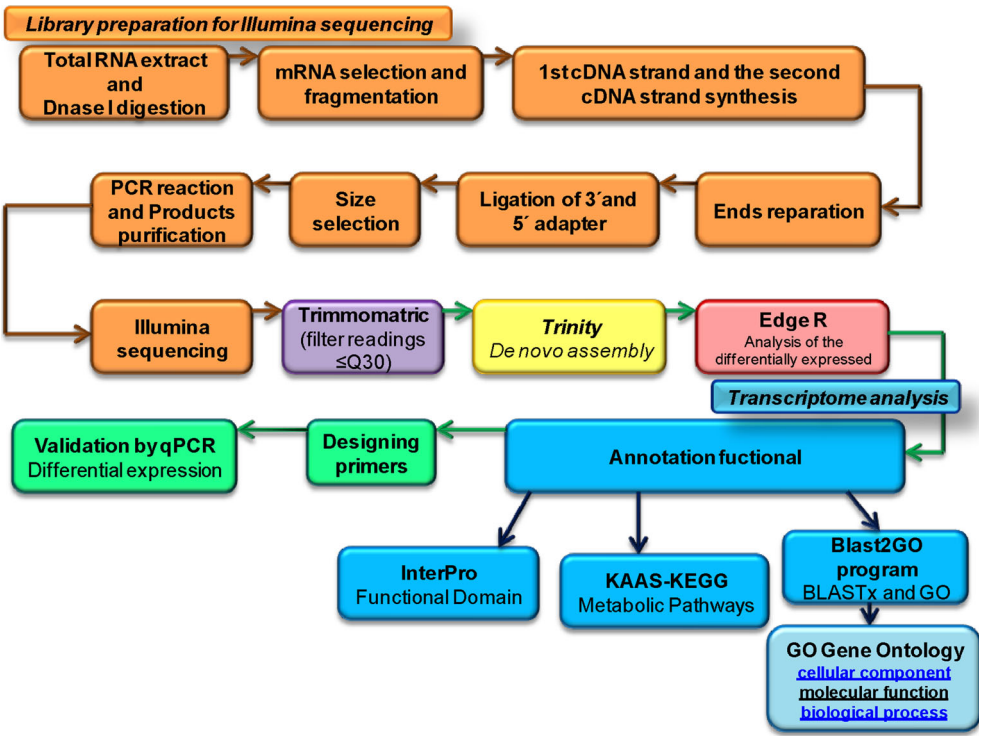

Fig. 1 Workflow diagram for transcriptome sequencing, assembly and analysis

with 300 cycles in accordance with the manufacturer's instructions. After filtering low-quality reads using default parameters of the Trimmomatic program (Bolger et al. 2014), the remaining high-quality reads were assembled using the default parameters of the Trinity program developed at the Broad Institute and the Hebrew University of Jerusalem (Grabherr et al. 2011). This software is specifically designed for assembling de novo transcriptomes. All transcriptome data were subsequently registered in DRYAD (accession number doi:10.5061/dryad.j970q). The assembled contigs of each treatment were annotated using the non redundant protein (Nr) NCBI database and InterProScan database. Data were further analyzed using the BLAST2GO software suite (http://www.BLAST2go.org) (Blüthgen et al. 2005; Götz et al. 2008) to predict functions of individual ESTs assigning Gene Ontology terms (Ye et al. 2006). Data were also analyzed with the KAAS- KEEG Automatic Annotation Server (Kanehisa and Goto 2000; Moriya et al. 2007; Kanehisa et al. 2008).

Identification of differentially expressed genes

Sequence reads were used to generate a single Trinity hybrid assembly (Grabherr et al. 2013) based on combining all reads across both treatments (T1 and T2). Transcript levels were measured as FPKM (Fragments Per Kilobase of exon model per Million mapped reads) for differential gene expression analyses (http://jura.wi.mit.edu/bio/education/hot_topics/RNAseq/RNA_Seq.pdf); these data were normalized using the TMM method (Robinson and Oshlack 2010). Statistical comparisons between the different transcriptomes were conducted using EdgeR Bioconductor software (Robinson et al. 2010, 2011) in accordance to the Trinity instructions. Transcripts were considered differentially expressed in a given library when the p-value was less than 0.05.

Differential expression validation by quantitative PCR

Quantitative PCR (qPCR), to validate differential expression, used the same total RNA pools of each treatment that were used for MiSeq-Illumina sequencing. First strand cDNA was synthesized from 2 μg of total RNA using a High Capacity cDNA Reverse Transcription (Invitrogen, USA) kit, in accordance with the manufacturer's protocol. Primers were designed using Primer3Plus software (http://biotools.umassmed.edu/cgi-bin/primer3plus/primer3plus.cgi). Seven candidate transcripts related to immune response and lipid metabolism were selected for validation by qPCR: sam domain and hd domain-containing protein 1 [comp8719_c0_seq1], interleukin enhancer-binding factor 2 homolog [comp8600_c0_seq3], solute carrier family 7 (cationic amino acid transporter) [comp10896_c0_seq3], platelet-activating factor acetylhydrolase [comp10353_c0_seq12], serine palmitoyltransferase 1 [comp10732_c0_seq1], elongation factor-1 alpha [comp7540_c0_seq1], and fatty acid synthase [comp45386_c0_seq1] (Table 4). The shrimp beta-actin gene was used as a constitutively expressed control (Cheng et al. 2005); (Chen et al. 2014). Quantitative PCR and data analysis were performed using a LightCycler 480 System (Roche). The qPCR reaction mixture (10 μL) contained

2× Power SYBR Green PCR Master mix, 0.5 μL of each forward and reverse primer, and 2.5 μL of template cDNA. Amplifications were performed under the following conditions: 1 cycle of 95 °C for 2 min; 35 cycles at 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min; and a final extension at 72 °C for 5 min. Receptor mRNA relative expression was measured using the method $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001). The relative expression of the seven target genes in treated (T2 *U. clathrata*) vs control shrimp (T1) was normalized to an endogenous reference gene (beta-actin) and expressed as the Normalized Target Gene Expression Level = $2^{-\Delta\Delta Ct}$, where ΔΔCt is defined as the difference between *ΔCt* of treated shrimp and *ΔCt* of control shrimp, and the *ΔCt* is defined as the difference between *Ct* target gene and *Ct* of reference gene (Brink et al. 2000). Serial dilutions from one of the samples were made to validate the efficiency of the qPCR assay and were tested with the oligonucleotides. The results were analyzed in a calibration curve considering that the regression value must be close to 1 and the slope close to −3.32 (Wong and Medrano 2005). Each qPCR reaction was carried out in triplicate.

Results and discussion

Sequencing and de novo assembly

As described in the aforementioned methods, libraries from cDNA synthesized from mRNAs pools of each treatment (T1 and T2) were sequenced using an Illumina MiSeq, resulting in 7, 706,527 of total raw reads. After evaluating the reads using the Trimmomatric trimming program and removing adaptors, a total of 6,591,856 cleaned reads were obtained. The GC content, ≥Q30, and unknown bases (N) were of 48.00, 78.90, and 0.00 %, respectively (Table 1). In the de novo assembly, 37, 078 transcripts were generated for (T1) with a 666-bp average length, and a N50 value of 1007 bp; for (T2), 31,615 transcripts were generated with a 592-bp average length, and a N50 value of 800 bp (Table 2). This last result shows us that our transcriptome

Table 1 General information of the transcriptome from T1 and T2 shrimp

Dataset name	T1 control diet	T2 control diet + *Ulva*	Total (T1 + T2)
Total raw reads			7,706,527
Total pass filter reads	3,855,180.00	2,736,675.00	6,591,856
% Total pass filter reads	57.4707	40.7967	98.2674
≥Q30 percentage	78.9	78.9	78.9
N50 (bp)	1007	800	903
% GC	48	48	48
% N	0	0	0

Table 2 General information of the *de novo assembly* from T1 and T2 shrimp

Dataset name	T1 control diet	T2 control diet + *Ulva*	Total (T1 + T2)
Total contigs	37,078	31,615	68,693
N50 (bp)	1007	800	903
Average length (bp)	666	592	629

database has acceptable coverage and the data greatly enrich existing information about the *L. vannamei* transcriptome.

Functional annotation

Functional annotation of transcript sequences was performed using BLASTx, along with the non-redundant protein NR sequence database of the NCBI (E-value threshold of 1e-5) (Guo et al. 2013) and analyzed using the Blast2GO program. A total of 15,861 transcripts for T1 (42.77 %) and 14,246 for T2 (45.06 %) were annotated in accordance to their homolog function (Table 3). Results on the distribution showing the best matches for each sequence are given in Fig. 2. Annotated unigenes showed matches with unigenes of *Daphnia pulex* (12 %), *Tribolium castaneum* (6 %), *Pediculus humanus* (5 %), *Branchiostoma floridae* (3 %), *Crassostrea gigas* (3 %), *Ixodes scapularis* (3 %), *Nasonia vitripennis* (3 %), *Capitella teleta* (3 %), followed by *Litopenaeus vannamei* (3 %). Our results agree with those of Guo et al. (2013) and Xiaohan Chen et al. (2013b), indicating that mostly all or the majority of unigenes are similar to those of *Daphnia pulex*, *Tribolium castaneum*, and *Pediculus humanus*; this might be attributed to the close phylogenetic relationship to these species, for which a great deal of genetic information is available over all of the annoted genes presented. On the other hand, the remaining annotated unigenes show certain similarity with other species, a fact which might be attributed to the relatively limited amount of the *L. vannamei* protein sequences available in the database.

Gene ontology (GO) analysis was performed using the Blast2GO program (Blüthgen et al. 2005; Götz et al. 2008) to determine the functional categories (Ye et al. 2006) represented by the *L. vannamei* transcriptome. The transcriptome comparison between T1 and T2 showed high similarity between transcript distributions in the three GO categories. We were able to observe that 18,682 (50.40 %) and 15,929 (50.30 %) of transcripts corresponded to biological processes, 14,466 (39.03 %) and 12, 335 (39.01 %) to cellular components, and 7980 (21.25 %) and 6804 (21.52 %) to molecular functions classified in 49 subcategories (Fig. 3). With regard to the biological processes category, the two subcategories that were most represented were: organic substance metabolic processes and primary metabolic processes. Concerning the cellular components, the most represented

Table 3 Summary of the annotations of *Litopenaeus vannamei* unigenes

	Number	
	T1	T2
All contigs	37,078 (100.00 %)	31,615 (100.00 %)
All annotated unigenes	23,510 (63.41 %)	20,901 (66.11 %)
Annotated using Nr database	15,861 (42.77 %)	14,246 (45.06 %)
Annotated using KEGG database	9649 (26.02 %)	8655 (23.37 %)
Annotated using GO database	23,733 (64.00 %)	20,824 (65.86 %)

subcategories were extracellular space components and cellular parts. Lastly, for the molecular function category, the most represented subcategories were binding function and catalytic activity. These subcategories were the most represented for both treatments in general, the functional transcript distributions were extremely similar between the two experimental groups of shrimp, and the number of transcripts expressed in each GO category was not significantly affected by *U. clathrata* consumption.

Subsequently, sequences were annotated using the KAAS-KEEG Automatic Annotation Server (Kanehisa and Goto 2000; Moriya et al. 2007; Kanehisa et al. 2008), with the purpose of determining the described metabolic pathways and homologue functions. Using KAAS-KEEG annotation, we characterized 9649 (26.02 %) and 8655 (23.37 %) transcripts, respectively in each treatment (T1 and T2).

Identification of differently expressed transcripts

A total of 396 differently expressed transcripts were identified between the two treatments (high expression and low

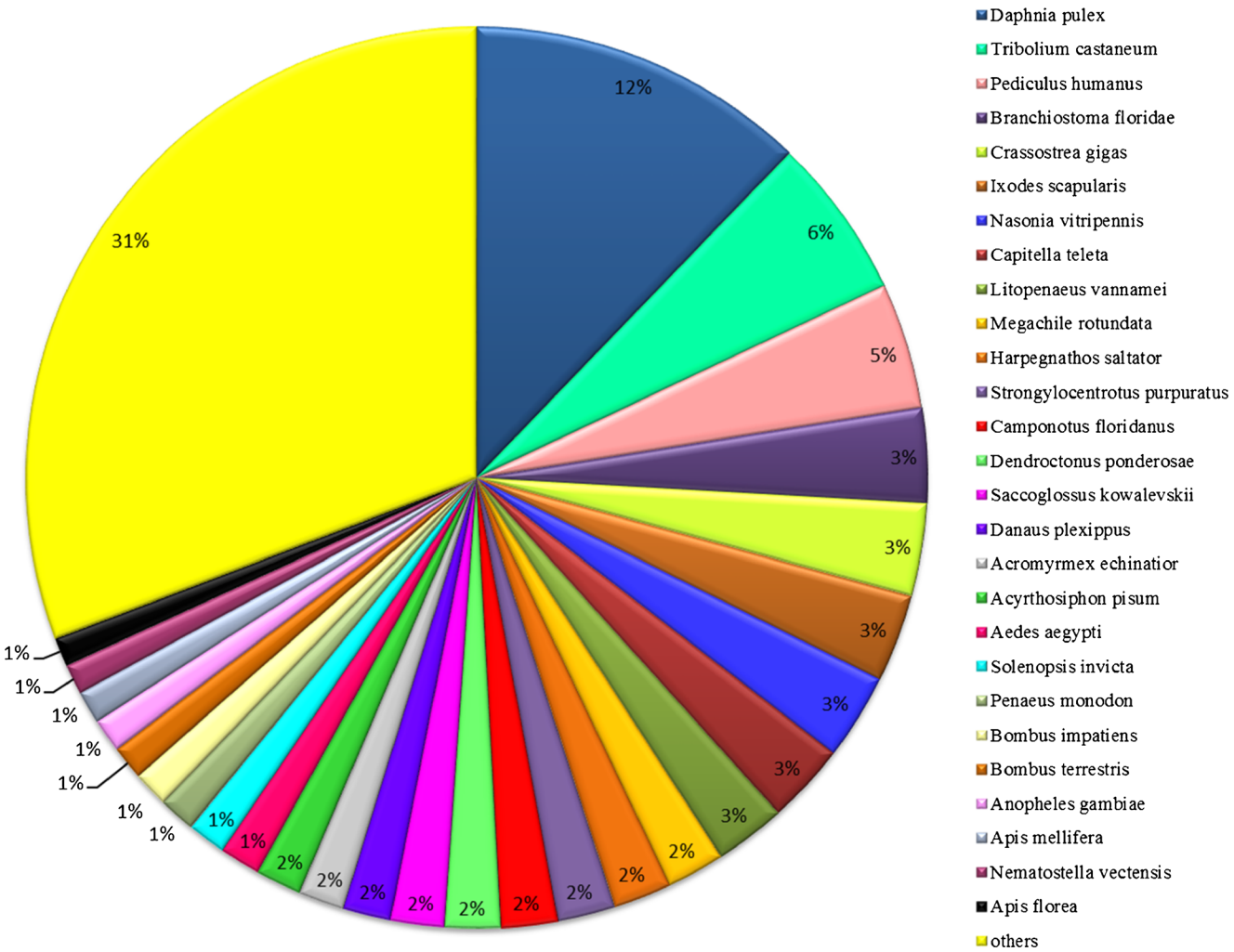

Fig. 2 Species distribution of the BLASTX results

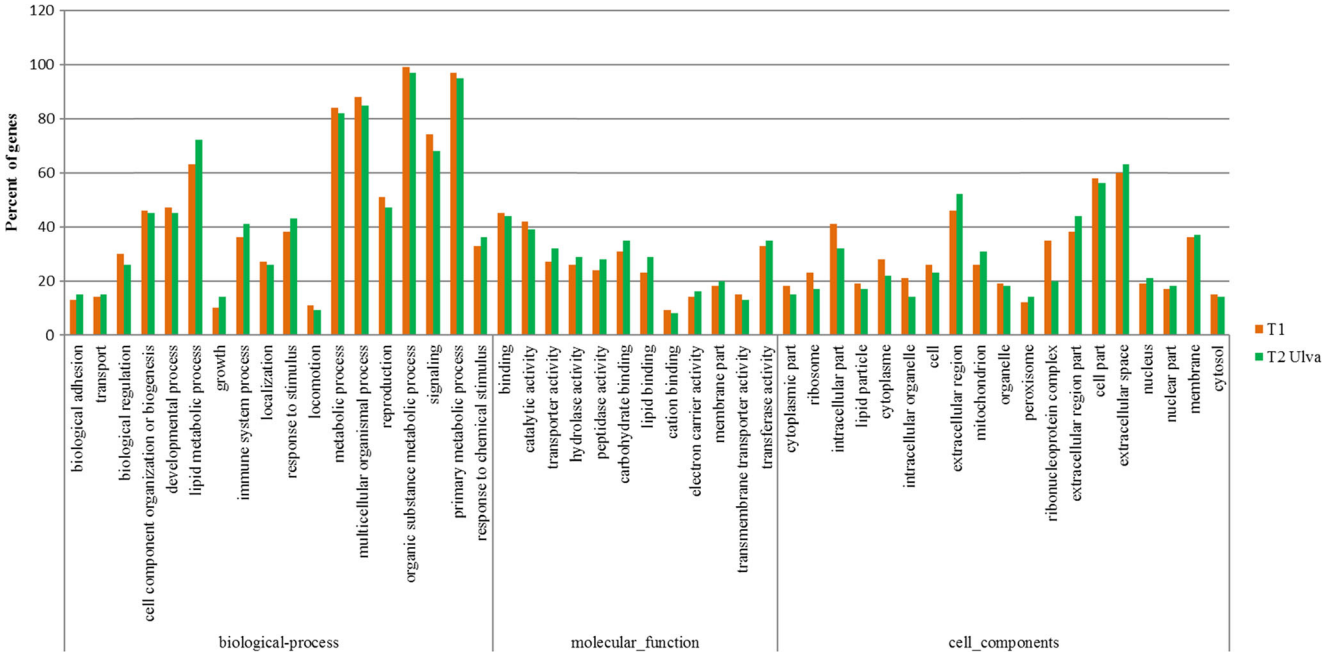

Fig. 3 Gene ontology (GO) classification of putative functions of unigenes from T2 CD + Ulva and T1 CD (control) *L. vannamei*

expression). For functional annotation, all the differentially expressed transcripts were annotated in the following gene databases: BLASTx, InterPro, GO, and KAAS-KEEG Automatic Annotation Server (Kanehisa and Goto 2000; Moriya et al. 2007; Kanehisa et al. 2008) and using the Blast2GO program (E-value threshold of 1e-5) (Guo et al. 2013). Of this total, 347 (87.4 %) were annotated in BLASTx, 284 (71.7 %) by GO, and 319 (80.5 %) by InterPro. An average 87 % of differentially expressed transcripts were annotated with the different databases; nevertheless, 13 % had low sequence homology with the sequences that were already known through public databases. These data matched with the aforementioned data presented by Zeng et al. (2013) and by Chen et al. (2013b). In the same way, they obtained 80 % similarity with currently reported transcripts. In contrast to these authors who obtained about 1300 differentially expressed transcripts, we observed only 396 differentially expressed transcripts. This may be due to the fact that the effector that was used in our study for inducing differential expression was the diet supplementation with *Ulva*. It is important to take into account that the animals were always under the same conditions (stress, health, nutritional, etc.); meanwhile, in the aforementioned studies, the effector which produced changes in gene expression was viral infection (WSSV in the article of Chen et al. 2013b and TSV in Zeng et al. 2013), which is obviously a more drastic effector as it causes great differences between healthy and diseased animals.

The relative expressions of seven candidate transcripts involved in immune response and lipid metabolism were determined in hepatopancreas by qPCR. Results showed that all these transcripts were significantly up-regulated in response to the consumption of *U. clathrata* as a dietary supplement (T2). The relative expression of T2 (*Ulva*) to T1 (control) was: 1.3-fold change of sam domain and hd domain-containing protein 1, 0.42-fold change of interleukin enhancer-binding factor 2 homolog, 0.60-fold change of solute carrier family 7 (cationic amino acid transporter), 0.47-fold change platelet-activating factor acetylhydrolase, 0.54-fold change of serine palmitoyltransferase 1, 0.49-fold change of elongation factor-1 alpha, and 22.7-fold change of fatty acid synthase. These results also further confirmed the reliability of RNA-seq and the accuracy of the Trinity assembly. It has been demonstrated that massive parallel sequencing technologies are a competent method that can be employed for detecting differentially expressed transcripts (Marioni et al. 2008).

KAAS- KEGG analysis of the differentially expressed transcripts

All differentially expressed transcripts were assigned to reference canonic pathways in the KAAS-KEEG Automatic Annotation Server with the purpose of establishing modified metabolic pathways. The top 15 pathways are presented in Fig. 4. The distribution of high-expression or low-expression transcripts between treatments was different: T1 presented the highest quantity of high-expression transcripts in the "Metabolic pathways," "Biosynthesis of secondary metabolites," "Microbial metabolism in diverse environments," "Carbon metabolism," "Glycerolipid metabolism," "Glycerophospholipids metabolism," "Ether lipid metabolism," "Amino sugar and nucleotide sugar metabolism," "Pathways in cancer," "MicroRNAs in cancer," "Influenza A," and "Pancreatic secretion" pathways; meanwhile, in T2, the high-expression transcripts were found

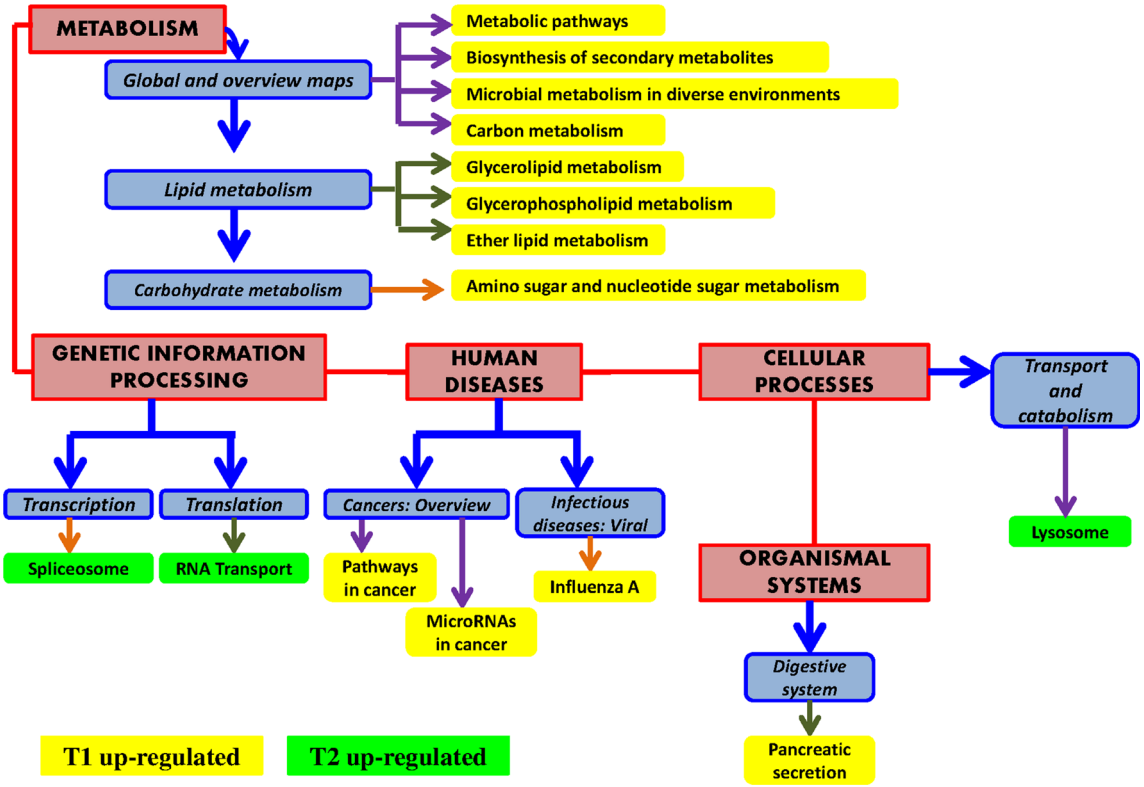

Fig. 4 KEGG analysis of up- and down-regulated genes

in the "Spliceosome," "RNA transport," and "Lysosome" pathways; this indicates some changes in the physiological state of shrimp which consumed *U. clathrata* (Table 4).

Candidate genes involved in the lipid metabolism and the immune response of *L. vannamei*

Concerning the metabolic pathways associated with immune response, the following pathways were affected: toll-like receptor signaling pathway, apoptosis, MAPK signaling pathway, melanogenesis, drug metabolism - cytochrome P450, natural killer cell-mediated cytotoxicity, and TNF signaling pathways (Table 5) (Cohen 1993; Dobrovolskaia et al. 2003; Andrade et al. 2004; Yang et al. 2008; Barakat et al. 2009). A few studies have reported that dietary supplementation with algae meals or extracts might improve the immune response, resistance, and survival of shrimp challenged with bacteria or viruses due to the presence of certain immune-stimulating compounds such as

Table 4 List of primers used in the real time PCR assay for relative quantification of genes

Shrimp gene			Primer sequence (5′-3′)	TM °C
Sam domain and hd domain-containing protein 1	F	5′	CAGTGTTCGAAACGGTTGTG	59.6 °C
	R	3′	AGGTTTCTGAAGCAGCTTGG	60.2 °C
Interleukin enhancer-binding factor 2 homolog	F	5′	TTGTGGTGATCCTCAAGACG	59.7 °C
	R	3′	TCATCTGGGAGCATTGTGAG	59.8 °C
Solute carrier family 7 (cationic amino acid transporter)	F	5′	ACTCTTTGCAGGGATCATGG	60.1 °C
	R	3′	TCCCCATCTTCATCTTCAGC	60.2 °C
Platelet-activating factor acetylhydrolase	F	5′	TGCTGTAGATGGAGCGATTG	60.0 °C
	R	3′	AACTGGCAAACAGGAAGTGC	60.3 °C
Serine palmitoyltransferase 1	F	5′	TGTTTCACTGGGCTGATGTC	59.7 °C
	R	3′	TGACCTTGTTGCAGAGTTGC	60.0 °C
Fatty acid synthase	F	5′	GTACCCATTGCCAGAGCAGT	60.1 °C
	R	3′	GCAGTTCAACTCGCTCAACA	60.2 °C
Elongation factor-1 alpha	F	5′	GGCTGCCTGAGAATCAAAAG	60.0 °C
	R	3′	TTCTCCTTGCCCATGGTTAC	59.9 °C

Table 5 Some candidate genes involved in immune response and lipid metabolic procesos of *L. vannamei*

Category or gene ID	Homologous function[a]
Toll-like receptor signaling pathway	
comp9997_c0_seq1	Mitogen-activated protein kinase kinase 1
Apoptosis	
comp10789_c0_seq4	Baculoviral IAP repeat-containing protein 2/3
MAPK signaling pathway	
comp9997_c0_seq1	Mitogen-activated protein kinase kinase 1
Melanogenesis	
comp9997_c0_seq1	Mitogen-activated protein kinase kinase 1
comp9273_c0_seq4	Guanine nucleotide-binding protein G(i) subunit alpha
Drug metabolism - cytochrome P450	
comp10988_c0_seq1	Glucuronosyltransferase
Natural killer cell mediated cytotoxicity	
comp9997_c0_seq1	Mitogen-activated protein kinase kinase 1
TNF signaling pathway	
comp9997_c0_seq1	Mitogen-activated protein kinase kinase 1
comp10789_c0_seq4	Baculoviral IAP repeat-containing protein 2/3
Ether lipid metabolism	
comp8505_c0_seq1	Ethanolaminephosphotransferase
comp5464_c0_seq1	Secretory phospholipase A2
comp10353_c0_seq12	Platelet-activating factor acetylhydrolase
comp10841_c0_seq3	Phospholipase D1/2
Fat digestion and absorption	
comp5464_c0_seq1	Secretory phospholipase A2
comp8774_c0_seq2	Pancreatic triacylglycerol lipase
comp8223_c0_seq1	Pancreatic lipase-related protein 2
Glycerophospholipid metabolism	
comp8026_c0_seq1	Glycerol-3-phosphate dehydrogenase (NAD+)
comp5501_c0_seq1	Glycerol-3-phosphate *O*-acyltransferase 1/2
comp8505_c0_seq1	Ethanolaminephosphotransferase
comp5464_c0_seq1	Secretory phospholipase A2
comp10841_c0_seq3	Phospholipase D1/2
comp11062_c0_seq1	Lysophospholipase III
Fatty acid degradation	
comp11173_c0_seq2	Aldehyde dehydrogenase (NAD+)
comp11057_c0_seq2	Acyl-CoA oxidase
Alpha-Linolenic acid metabolism	
comp11057_c0_seq2	Acyl-CoA oxidase
comp5464_c0_seq1	Secretory phospholipase A2
Fatty acid biosynthesis	
comp45386_c0_seq1	Fatty acid synthase, animal type
Linoleic acid metabolism	
comp5464_c0_seq1	Secretory phospholipase A2
Biosynthesis of unsaturated fatty acids	
comp11057_c0_seq2	Acyl-CoA oxidase
Arachidonic acid metabolism	
comp5464_c0_seq1	Secretory phospholipase A2

[a] The functions of homologous gene

fucoidan, alginates, laminarins, and carragenans (Selvin et al. 2004; Cheng et al. 2008; Cruz-Suárez et al. 2009). At a molecular level, it has been demonstrated that the addition of sulfated polysaccharides of *Ulva* (*Enteromorpha*) *prolifera* stimulates the production of macrophages and increases cytokine expression (Kim et al. 2011). Other studies have shown that polysaccharides and extracts from marine algae might have a certain influence on the immune response genes (Leiro et al. 2007; Reilly et al. 2008; Yoon et al. 2009; Bahar et al. 2012; Kandasamy et al. 2012).

The following modified metabolic pathways were associated with lipid metabolism: ether lipid metabolism, fat digestion and absorption, glycerophospholipid metabolism, fatty acid degradation, alpha-linolenic acid metabolism, fatty acid biosynthesis, linoleic acid metabolism, biosynthesis of unsaturated fatty acids, or arachidonic acid metabolism (Table 5). These results confirm the effect at a molecular level on the lipid metabolism and agree with previously reported studies (Maeda et al. 2005; Seo et al. 2013). These studies also demonstrated that *U. clathrata* had significant effects on lipid composition of different fishes and terrestrial animal species (Nakagawa et al. 1987; Nakagawa 1997; Wong et al. 1999; Pengzhan et al. 2003; Ortiz et al. 2006; Carillo et al. 2008; Ergün et al. 2008). In shrimp, *U. clathrata* promotes the production of certain 18 carbon polyunsaturated fatty acids (PUFAs) (Kumari et al. 2010), as well as favoring an increase in the content of 18:0, 18:3n3, 20:5n3 (Ortiz et al. 2006; Carillo et al. 2008; Peña-Rodríguez et al. 2010). In contrast, it has been demonstrated that fucoidan treatment (polysaccharide extracted from brown seaweed) affects adipogenesis in 3T3-L1 cell lines (Kim et al. 2010) as it suppresses the adipocites differentiation and inhibits the expression of adipogenic transcription factors α (C / EBPAα), γ (PPARγ) and AP2. In the same way, the inclusion of *Ulva linza* and *Lessonia trabeculata* in diets for rats significantly reduces the content of intra abdominal fat, cholesterol, and triglyceride serum levels in healthy animals, as well as in animals showing signs of metabolic syndrome, and at the same time, there is a reduction in catalase gene expression in animals with metabolic syndrome (Ramirez-Higuera et al. 2014). In our study, it was possible to demonstrate these modifications (at genomic level) in the lipid metabolism of shrimp fed with *Ulva*.

Conclusion

In this study, massive parallel sequencing of RNA has been employed with the intention of examining the *L. vannamei* transcriptome in response to the consumption of *U. clathrata* as a dietary supplement. This is considered a first work at the molecular level regarding nutrigenomics related to the consumption of marine algae by *L. vannamei*. Differentially expressed genes were detected between both nutritional conditions, and the transcriptional reactions mainly described the immune response pathway, lipid metabolism, stress and stimulus response, and oxidation–reduction processes. The identified genes and/or the described metabolic pathways suggest that *Ulva* consumption modified the molecular mechanisms involved in shrimp immune system, as well as in the deposition and movement of different lipid classes in the tissues of shrimp.

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