Physiological and Biochemical Responses of *Cyclina sinensis* on Different Concentrations of Dietary Microalgae

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Introduction

Algae are an important component of the aquatic ecosystem and one of the key factors that determine the success of filter-feeding shellfish farming. Algae in ponds are not only an important source of initial creativity in the water body, but also the main bait for filter-feeding shellfish. When some planktonic algae, protozoa or bacteria in seawater accumulate to a certain critical point under the influence of adverse environmental factors, the ecological balance of the ocean is destroyed, and phytoplankton proliferates explosively, resulting in disasters.

Shellfish have a well-developed filter-feeding system and a strong water-filtering ability, which can feed on phytoplankton and suspended particulate matter in the water, thereby affecting the water quality. The clam *Cyclina sinensis* are widely distributed in the coastal beaches and estuaries of China, Japan and North Korea, and have been widely cultivated and proliferated in China. In the actual production of artificial culture, the concentration of algae in the pond will seriously affect the food intake ability of clams. Excessive growth of algae leads to eutrophication in water, fish and shellfish and other organisms slowly die, and filter-feeding shellfish causes human harm after ingestion.

In this paper, the common tidal flat culture of shellfish *Cyclina sinensis* constricta along the coast of China were used as the research objects. The culture experiments were carried out by setting different microalgae feed concentrations. The effects of different feed concentrations on the growth performance, digestion, immunity, and respiratory metabolism-related enzyme activities of *C.sinensis* was studied. Used the Illumina Hiseq sequencing platform for hepatopancreas of *C.sinensis* under the stress of relevant food concentration, dig out differentially expressed genes and pathways under the stress of different food concentrations, To provide scientific evidence for the growth, physiological and biochemical performance of filter-feeding shellfish in different eutrophic water bodies.

Methods

1. Experimental animals and sample collection

Samples of *Cyclina sinensis* were purchased from Lianyungang Zhongchuang Aquaculture Co., Ltd., and fed in water tanks (length width depth=100cm 100cm 40cm) at Breeding base in Ganyu District, Lianyungang City, Jiangsu Province, China. The concentrations of algae in T1, T2, T3, T4 and T5 grops were 5×10^5, 1.25×10^6, 1.30×10^7, 1.25×10^8 and 1.5×10^9cell/L respectively. The algae were tested using the AP110 chlorophyll instrument, cultured in the same area and at the same water depth, with an aerated pump, aerated daily and seawater changed every other day. Experimental samples were collected after 90 days.

2. Sample and data analysis

2.1. The growth indicators were calculated using the following parameters:

Survival rate = $S_t / S_0 \times 100\%$;

Average daily shell height increase = $(Sh_t - Sh_0)/t$

Average daily shell length growth = $(Sl_t - Sl_0)/t$

Average daily increase in individual mass = $(Bm_t - Bm_0)/t$

Fatness = dry meat weight / dry shell weight * 100%

Hepatopancreas index = Hepatopancreas wet weight/body wet weight * 100%

Specific growth rate = SGR = 100% * $(\ln W_t - \ln W_0)/t$

In each equation: S_0, Sh_0, Sl_0 and Bm_0 are the number, the height, the length and the individual mass of clams at the beginning of the test and at the end of the test,respectively; $\ln W_t$ and $\ln W_0$, the individual mass at the end of the trial (g) and the individual mass of the initial trial (g), respectively, and t is the number of trial days (d).

2.2. Activities of enzymes related to immunity, digestion and respiratory metabolism of *C. sinensis*

The activities of Total Superoxide Dismutase Enzyme (T-SOD), Acid Phosphatase (ACP), Lysozyme (LZM), Amylase (AMS), Lipase (LPS), Trypsin, Lactate Dehydrogenase (LDH), Succinate Dehydrogenase (SDH) were measured using commercial kits according to the protocols(Nanjing Jiancheng Bioengineering Institute).

2.3. Transcriptome analysis of the hepatopancreas of *C. sinensis* serrata in response to different food concentrations

2.3.1 Sample extraction and high-throughput sequencing

2.3.2 Sequencing data analysis and transcript assembly

2.3.3 Functional annotation and enrichment analysis

2.3.4 Identification and signaling pathway analysis of differentially expressed genes (DEGs)

2.3.5 Quantitative real-time PCR (qRT-PCR) verification

2.4. Statistical analysis

Results are presented as mean ± standard error of the mean (SEM) calculated by GraphPad Prism 8.0 software.

Results

Biological data of *C. sinensis* under different food concentrations. Table.1

Table 1. Biological characteristic of *C. sinensis* in different food concentration

density component (cell/L)	SLGR/%	SHGR/%	SR/%	IC/%	HSI/%
T1	0.25 ± 0.002^a	0.26 ± 0.002^a	87^a	4.2^a	1.6 ± 0.005^b
T2	0.24 ± 0.002^a	0.25 ± 0.002^a	84^a	4.0^b	1.8 ± 0.006^b
T3	0.13 ± 0.002^b	0.13 ± 0.001^b	61^b	1.4^e	1.9 ± 0.007^{ab}
T4	0.09 ± 0.002^b	0.11 ± 0.002^b	57^b	2.3^c	2.4 ± 0.008^a
T5	0.06 ± 0.002^b	0.08 ± 0.001^b	41^c	2.0^d	1.8 ± 0.007^b

Note: "a、b、c" indicate significant differences among groups at food concentration ($p < 0.05$)

Impacts of different food concentrations on the immunity, digestion and respiratory capability. Fig.1, Fig.2, Fig.3.

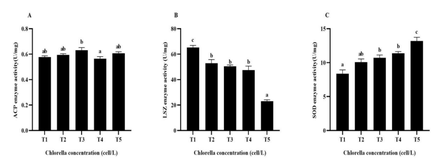

Fig. 1 Immune-related enzyme activities in the hepatopancreas the *C. sinensis* under different concentrations of Chlorella.

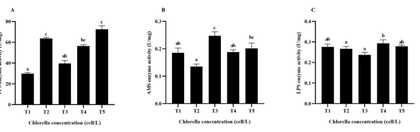

Fig. 2 Digestive-related enzyme activities in the hepatopancreas the *C. sinensis* under different concentrations of Chlorella.

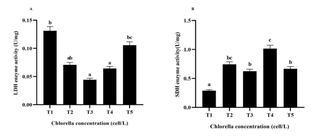

Fig. 3 Respiration-related enzyme activities in the hepatopancreas.

Transcriptome results

After adaptor sequences and low-quality reads were removed, 211,645,85 clean reads, 214,747,69 clean reads, and 244,489,80 clean reads were obtained respectively from the T1, T3 and T5 groups of *C. sinensis* transcriptomes(Table 3). Based on the annotation of NR database, *Mizuhopecten yessoensis* (31%) ranked number one in all species followed by *Crassostrea gigas* (18%), *Crassostrea virginica* (16%), *Lottia gigantea* (6%), *Lingula anatina* (3.00%), *Aplysia californica* (3%), *Octopus bimaculoides* (2%), *Biomphalaria glabrata* (2%) and *Branchiostoma belcheri* (1%) (Fig. 4).

Table 3 Transcriptome statistics

Sample	Total Reads	Clean Reads	GC%	Q30	Genome map Rate
T1	42329170	21164585	40.44	92.89	76.66
T3	42949538	21474769	40.45	92.72	74.70
T5	48897960	24448980	40.34	92.59	74.44

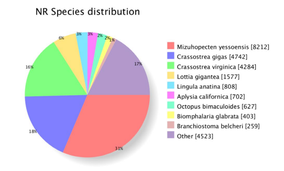

Fig. 4. Species distribution results of a similarity research of unigenes against NR database.

The DEG results showed that compared to the T1 group, 1244 upregulated and 1753 downregulated genes were observed in the T3 group, and 1881 upregulated and 2158 downregulated genes were observed in the T5 group; 1820 upregulated and 1541 downregulated genes were observed in the T5 group compared to T3 group (Fig. 5.).

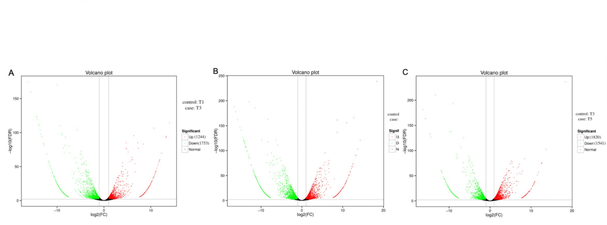

Fig. 5. A volcano plot of differentially expressed genes (DEGs). Red spots indicate significant up-regulated genes, while green spots indicate significant down-regulated genes. (A) T1 vs T3, (B) T1 vs T5, (C) T3 vs T5

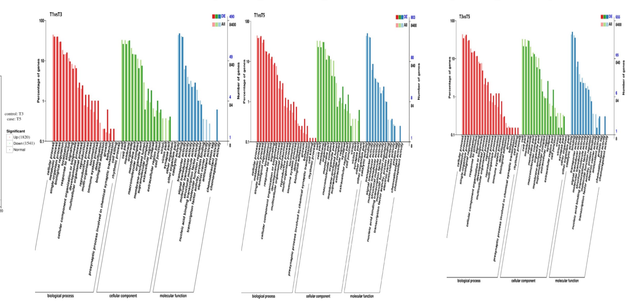

Fig. 6. Differential gene ontology (GO) pathway enrichment column (A) T1 vs T3, (B) T1 vs T5, (C) T3 vs T5

The GO terms were divided into 3 functional categories.Fig.6

The KEGG pathways were annotated into 6 biochemical pathways. Fig.7

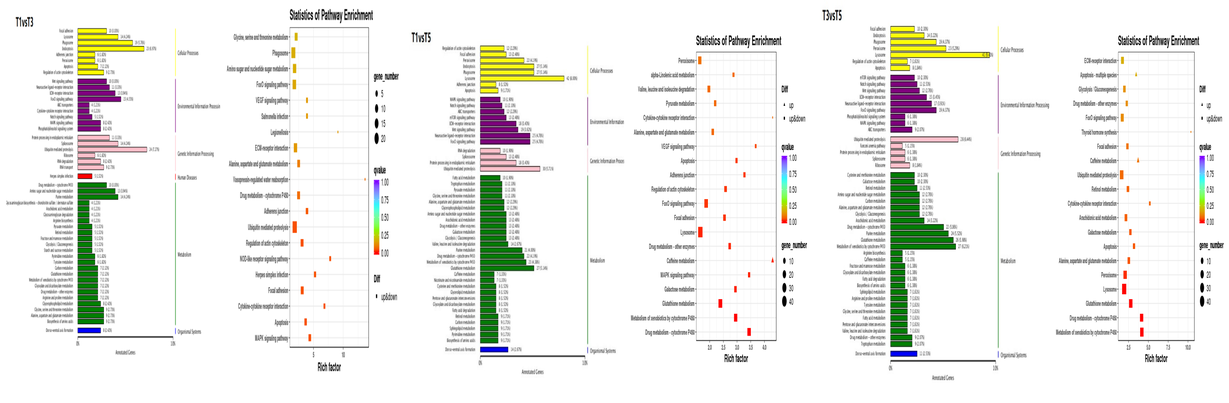

Fig. 7. Differential gene Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment column (A, C, E) and bubble (B, D, F) map. (A, B) T1 vs T3, (C, D) T1 vs T3, (E, F) T3 vs T5.

To validate the expression findings from deep sequencing, 12 DEGs involved in respiratory metabolism, digestion and growth in *C. sinensis* biological process were detected by qRT-PCR. Experimental results supported the reliability of the RNA-seq results (Fig. 8).

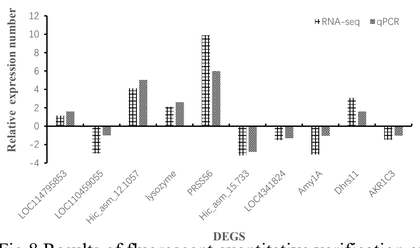

Fig.8 Results of fluorescent quantitative verification of differentially expressed genes

Conclusion

In conclusion, this study found that excessively high concentrations of microalgae bait will have a significant impact on the growth and survival, digestion and immune metabolism of tidal flat shellfish. In practice, the concentration of microalgae in the water environment should be avoided to exceed 5×10^5cell/L.

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