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Effects of elevated ammonium on the transcriptome of the stony coral *Pocillopora damicornis*

Chao Yuan, Zhi Zhou *, Yidan Zhang, Guangmei Chen, Xiaopeng Yu, Xingzhen Ni, Jia Tang, Bo Huang *

Key Laboratory of Tropical Biological Resources of Ministry of Education, Hainan University, Haikou, Hainan, People's Republic of China

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ABSTRACT

The survival of corals worldwide has been seriously threatened by eutrophication events concomitant with the increase in ocean pollution. In the present study, whole transcriptomes of the stony coral *Pocillopora damicornis* exposed to elevated ammonium were sequenced. A total of 121,366,983 pair-end reads were obtained, and 209,337 genes were assembled, including 42,399 coral-derived and 54,874 zooxanthella-derived genes. Further, a comparison of the control versus stress group revealed 6572 differentially expressed genes. For 1015 significantly upregulated genes, 24 GO terms were overrepresented, among which 3 terms related to apoptosis and cell death induction included one caspase, five bcl-2-like proteins, and two tumor necrosis factor receptor superfamily member genes. For 5557 significantly downregulated genes, the top 10 overrepresented terms were related to metabolism and signal transduction. These results indicate that apoptosis and cell death could be induced under elevated ammonium, suggesting that metabolic regulation and signal transduction might be involved in the reconstruction of the coral–zooxanthellae symbiotic balance in the stony coral *P. damicornis*.

1. Introduction

Reef-building stony coral is a fundamental pillar for biologically and economically important coral reef ecosystems (Yamashita et al., 2014). Stony corals can effloresce in oligotrophic tropical seas, depending on the elaborate coral–algal symbiosis. In this symbiosis, stony corals provide carbon dioxide for the photosynthesis of the engaged zooxanthellae, while zooxanthellae as symbionts supply photosynthetic products to stony corals (Shinzato et al., 2011). On the basis of this symbiotic relationship, coral reefs are known to be among the world's most productive ecosystems and contain a remarkable diversity of species growing on limited surfaces (Karlson et al., 2004). However, the survival and reproduction of stony corals have been threatened by environmental and anthropogenic aggressions in recent years (Abram et al., 2003; Cerrano et al., 2013; Feuillassier et al., 2014).

Recent global climate change and human activities have accelerated seawater temperature rise, ocean acidification, and eutrophication events; these conditions have ultimately threatened the survival of corals and the sustainability of existing coral reefs worldwide (Bellwood et al., 2004; Seemann et al., 2014). Rapid increase in the atmospheric carbon dioxide concentration, driven by global warming and ocean acidification, is likely to be a serious threat to coral survival (Hoegh-Guldberg et al., 2007). The corals growing under this stress condition are more susceptible to pathogenic microorganisms, which can lead to coral diseases and bleaching (Mydlarz et al., 2009; Weis, 2008). In addition, eutrophication is an easily overlooked factor that threatens coral survival. Eutrophication of coastal waters has recently emerged as a crisis because of dumping of domestic and industrial wastewater containing abundant nutrients directly into the sea (Cheevaporn and Menasveta, 2003; Fabricius, 2011; Fabricius, 2005). Large-scale chronic eutrophication obstructs the survival of corals and the sustainability of coral reef (Bell et al., 2014). Even worse, eutrophication has been considered as a major cause of decline in coral reef (Szmant, 2002).

Eutrophication can exert harmful effects on the survival and reproduction of stony corals; therefore, the effects of dissolved inorganic nutrients as important components of eutrophication have often been studied (Dubinsky and Stambler, 1996; Koop et al., 2001; Lam et al., 2015). Previous studies have reported that high levels of dissolved inorganic nitrogen and phosphorus could cause significant physiological changes in stony corals, including reduction in calcification, higher concentrations of zooxanthellae, and potentially higher rates of coral diseases (Bruno et al., 2003; Marubini and Davies, 1996; Stambler et al., 1994). It has been thought that exposure to dissolved inorganic nutrients alters the energy and nutrient transfer between zooxanthellae and coral, but do not directly inhibit the growth of individual coral colonies (Fabricius, 2005). Furthermore, eutrophication can affect the embryogenesis and ontogenesis of stony corals. High levels of inorganic nutrients can reduce fertilization success, egg viability, and polyspermic block in the coral *Platygyra acuta* (Lam et al., 2015); impair fertilization success and embryo development in the corals *Acropora longicyathus*

* Corresponding authors.
E-mail addresses: zhouzhi@hainu.edu.cn (Z. Zhou), huangbohb1@163.com (B. Huang).

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and *Goniastrea aspera* (Harrison and Ward, 2001); and reduce ciliary activity (motility) and settlement rate in the larvae of the coral *Diploria strigosa* (Bassim and Sammarco, 2003). The effects of eutrophication on coral survival and reproduction have been ascertained at the individual and cellular levels, but their underlying molecular mechanisms remain unclear.

Pocillopora damicornis is a common reef-building stony coral found on tropical coral reefs throughout the Indo-Pacific region. In recent years, the next-generation sequencing technology has emerged as a cutting-edge approach for high-throughput sequence determination and has been applied to greatly enrich the transcriptome resources of corals (Moya et al., 2012; Polato et al., 2011; Shinzato et al., 2014; Sun et al., 2013; Vidal-Dupiol et al., 2014; Vidal-Dupiol et al., 2013). In the present study, the transcriptome response of the coral *P. damicornis* was investigated after exposure to elevated ammonium through the next-generation sequencing technology; this study could pave a new way to further understand the modulation mechanism of stress response and environmental adaption to eutrophication in stony corals. The purposes of this study were to (1) identify the transcripts from the coral *P. damicornis*, (2) determine the differentially expressed genes of the coral after exposure to elevated ammonium, and (3) investigate the possible coral physiological changes resulting from these differentially expressed genes to understand the potential molecular regulation mechanisms of eutrophication stress.

2. Materials and methods

2.1. Corals

Samples of the stony coral *P. damicornis* were collected from the coral reef in Wenchang, Hainan Province, China, and maintained in seawater at 28 °C for 2 weeks before processing.

2.2. Elevated ammonium treatment

To avoid the potential effect of interindividual variability and genetic background, the treatment experiment was performed using clones of the same *P. damicornis* isolate. Elevated ammonium seawater was prepared by adding ammonium chloride to filtered seawater, and the final concentration of ammonium chloride was 100 μmol L^{-1}. Coral nubbins were randomly divided into two groups: control (NH4_Control) and stress (NH4). The coral nubbins in the NH4_Control group were placed in 25-L tanks ($n = 2$ per tank) containing filtered seawater, while the coral nubbins in the NH4 group were placed in the elevated ammonium seawater. After 12 h, all coral nubbins were sampled with two biological repetitions in each group. To each sample, 1 mL Trizol reagent (Invitrogen, Carlsbad, CA) was added, and the sample was stored in liquid nitrogen immediately for RNA extraction.

2.3. Construction and sequencing of transcriptome libraries

Total RNA was isolated from each coral sample using Trizol reagent (Invitrogen) according to the manufacturer's protocol. The harvested total RNA was quantified by Nanodrop 2000 (Thermo Scientific) at 260/280 nm (ratio > 2.0), and its integrity was checked with Agilent 2100 Bioanalyzer (Agilent Technologies). The pair-end fragment library (2 × 100 bp) was constructed and sequenced on the Illumina Hiseq4000 platform according to the manufacturer's instructions (BGI, Shenzhen, China). The raw sequencing reads were submitted to NCBI Short Read Archive under accession number **SRR2757326**.

2.4. Assembly of coral transcripts

Raw reads obtained from Illumina sequencing were processed by the Fastx-Toolkit pipeline (http://hannonlab.cshl.edu/fastx_toolkit/index.html) to summarize data production, evaluate sequencing quality, and remove low-quality reads and adaptor sequences. The obtained clean reads from four libraries were merged to assemble the transcripts using the Trinity software (http://trinityrnaseq.github.io/) (Haas et al., 2013). The redundant transcripts were identified and removed using TGICL software (http://sourceforge.net/projects/tgicl/) (Pertea et al., 2003).

The coral and zooxanthella transcripts were further differentiated using the BLAST software. Because the genomes of the coral *Acropora digitifera* and the zooxanthella *Symbiodinium minutum* have been sequenced (Shinzato et al., 2011; Shoguchi et al., 2013), their protein sequences were downloaded and merged. After a protein BLAST database was constructed, the assembled transcripts were aligned to the protein database through BLASTX algorithm (evalu = 1−e5, max_target_seqs = 1). The transcripts whose encoded proteins shared high sequence similarity with *S. minutum* proteins were regarded as zooxanthella-derived transcripts, while the transcripts whose encoded proteins shared high sequence similarity with the proteins from *A. digitifera* were referred to as the transcripts derived from the coral *P. damicornis*. The coral transcripts and corresponding genes were used in the subsequent function annotation and reads mapping.

2.5. Identification of differentially expressed genes

The assembled coral transcripts served as the reference sequence in the reads mapping, and a GTF annotation file was constructed using a homemade Perl script. All pair-end reads were aligned using TopHat software. Cufflinks software was used to assemble transcripts, estimate their abundances, and identify the differentially expressed genes between the NH4_Control and NH4 groups (Trapnell et al., 2012). The RNA-Seq data were analyzed using an R package of CummeRbund.

2.6. GO enrichment of differentially expressed genes

Similar coding regions of the assembled coral transcripts were extracted, and their encoded proteins were then retrieved using TransDecoder software (http://transdecoder.github.io/). These transcripts and proteins were aligned by local BLASTX and BLASTP search to SwissProt and Uniref90 databases (max_target_seqs = 1). The potential domains of these proteins were predicted through a Pfam search. The obtained alignment results were further parsed by Trinotate software (http://trinotate.github.io/) for function annotation and GO term assigning.

After the identification of differentially expressed genes, the lists of significantly upregulated and downregulated genes were obtained. Their GO enrichment analysis was implemented by the hypergeometric test with a filter value of 0.01. The differentially expressed genes were selected as the test set, while all other genes were considered as the reference set. The significantly overrepresented and underrepresented GO terms were identified from the test set using BiNGO tool (Maere et al., 2005).

3. Result

3.1. Construction and sequencing of transcriptome libraries

In the present study, the RNA-seq method was used for the analysis of transcriptome from the stony coral *P. damicornis*. A total of four transcriptome pair-end libraries were constructed from the coral after the exposure to elevated ammonium seawater, including the NH4_Control and NH4 groups (two repeats for each group), and they were sequenced to the saturated level by Illumina Hiseq4000 platform to compare the gene expression abundance and difference. After filtering low-quality sequences and adaptor sequences, there were 121,366,983 pair-end reads with a length of 2 × 100 bp obtained from all the libraries, including 30,274,934 and 30,365,662 reads from the NH4_Control libraries, and 30,581,298 and 30,145,089 reads from the NH4 libraries.

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