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Holobiont transcriptome of colonial scleractinian coral *Alveopora japonica*

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ABSTRACT

Climate change rapidly warms the ocean and marine species often move northwards for suitable habitats. Stony coral, *Alveopora japonica*, is observed more frequently for the last few years in temperate sea like Jeju Island, South Korea. To understand the ecological consequences such as habitat formation and fate of this species in changing environment, unraveling the genetic makeup of the species is essential. We sequenced the transcriptome of the *A. japonica* holobionts using Illumina HiSeq2000 platform. *De novo* assembly and analysis of coding regions predicted 108,636 coding sequences consisted of the coral host and residing *Symbiodinium*. Homology analysis showed the gene contents from our assembly are comparable to other sequenced corals and Symbiodiniums. The reference assembly of *A. japonica* will be a valuable resource to study the ecological characteristics of this species in the marine benthic ecosystem.

1. Introduction

The colonial stony coral *Alveopora japonica* is a zooxanthellate scleractinian coral in the family Acroporidae (WoRMS, n.d.). Corals in the genus *Alveopora* are generally uncommon and native to the Indo-Pacific region. *Alveopora japonica* is distributed in a high-latitude area extending from southern Taiwan to Japan (Veron & Stafford-Smith, 2000) and is found in shallow, partly wave-washed rocky foreshores, nested among algae and soft corals (Dai, 2009) (Fig. 1). This species displays characteristic hermaphroditic brooding, with oocytes and spermatocytes developing on separate mesenteries of the polyp, and takes 3 years to reach sexual maturity (Harii et al., 2001). This coral is associated with a clade F *Symbiodinium*, which was identified by a phylogenetic reconstruction based on internal transcribed spacer 2 (ITS2) sequences (De Palmas et al., 2015).

The genus *Alveopora* is considered to have the highest bleaching response and is in the top 10 genera at risk of extinction in the western Indian Ocean (McClanahan et al., 2007). The natural habitat of this uncommon species is also highly likely to be reduced by a combination of factors, including coral removal and harvesting for the curio trade, for display in aquaria. Jeju Island is located in the southwest of the Korean peninsula (33°24′N, 126°32′E) and is characterized by a warm temperate climate, an effect of the Kuroshio Current (KC), which circulates from the tropical Philippines to subtropical Taiwan and into the temperate region of Japan. The KC is experiencing a rapid increase in seawater temperature in response to global climate change and warmed most rapidly from 1981 to 1998, when the surface temperatures rose by 1.5 °C (0.9 °C/decade, almost seven times the global rate) (Park & Oh, 2000). Because the KC is rapidly warming, some reef-building corals may expand their habitats northward in response to increasing temperatures, although the Jeju Island coast does not have an appropriate environment for coral reef formation (Denis et al., 2014; Yamano et al., 2011). This species may benefit from the recent increase in seawater temperature, and may shift from a kelp-forest habitat to a coral-dominated habitat, disrupting the *competitive interactions among benthic taxa around Jeju Island (Denis et al., 2014)*.

Comprehensive molecular studies help to understand the effect of habitat shifts on the organisms. A transcriptomic analysis using the high-throughput sequencing technology is now the standard procedure to determine an organism's responses to a different environment, including climatic changes (Veilleux et al., 2015; Maor-Landaw et al., 2017). Due to their obligative symbiotic relationship and difficulty of physical separation, the coral host and its endosymbiotic dinoflagellate, *Symbiodinium*, are often sequenced and analyzed simultaneously to provide the snapshot of the coral holobiont activities in a given environment (Shinzato et al., 2014). The aim of the present study was to establish a reference transcriptome for the subtropical coral *A. japonica* holobiont at the temperate latitude of Jeju Island and to prepare

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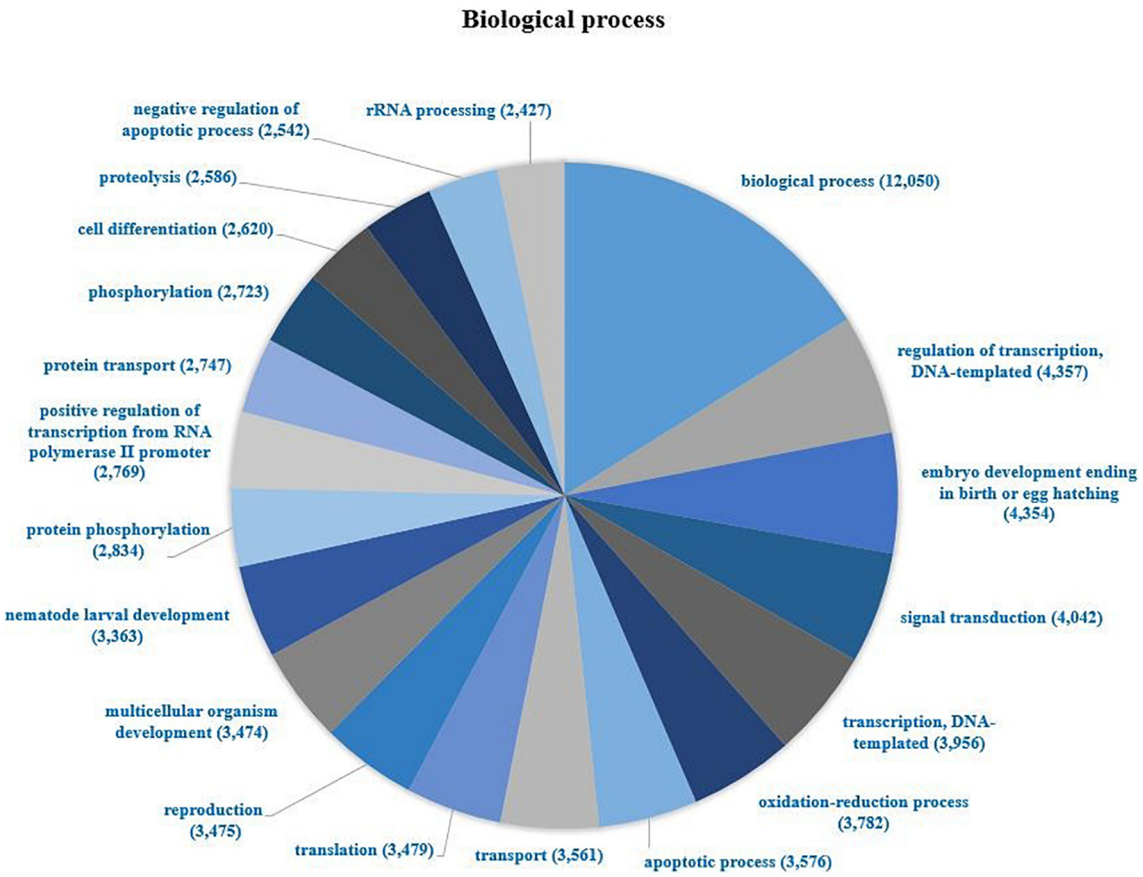

Fig. 1. Functional profile of *A. japonica* transcripts using GO Biological process.

Table 1
MIxS specifications of *A. japonica* transcriptome.

Item	Description
Investigation_type	Eukaryote
Project_name	Reference transcriptome of colonial scleractinian coral *Alveopora japonica*
Organism	*Alveopora japonica*
Classification	Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Cnidaria; Anthozoa; Hexacorallia; Scleractinia; Fungiina; Poritidae; Alveopora
Lat_lon	33.24N, 126.32E
Geo_loc_name	South Korea, Jeju island, Seogwipo
Collection_date	2016-03-05
Collector	Seonock Woo
Environment (biome)	marine benthic biome (ENVO:01000024)
Environment (feature)	sea shore (ENVO:00000485)
Environment (material)	sea water (ENVO:00002149)
Env_package	Water
Seq_meth	Illumina
Transcriptome_platform	HiSeq2000
Assembly_method	Trinity v2.3.2
Submitted_to_INSDC	Bioproject (PRJNA436760)
	Biosample (SAMN08631865)
	SRA (SRX3777227)
	GenBank (GGJR00000000)

baseline data for future studies of the effects of climate change on the movement, habitat selection, and distribution of this rare coral species at the molecular level.

2. Data description

2.1. Sample collection

Alveopora japonica coral colonies were collected at water depths of approximately 5–7 m near Seogwipo, Jeju Island, Korea, and directly frozen in liquid nitrogen immediately after collection (5th March 2016). The total RNA was extracted following the method described by Woo et al. (Woo et al., 2005). In brief, coral polyp tissues were pulverized in a mortar with liquid nitrogen. The polyp powder was then homogenized in 700 µl of lysis solution (35 mM EDTA, 0.7 M LiCl, 7% SDS, 200 mM Tris-Cl [pH 9.0]), and the RNA was extracted with 700 µl of water-saturated phenol. A one-third volume of 8 M LiCl was added to the retained aqueous phase, which was then stored at 4 °C for 2 h. The RNA was precipitated after centrifugation at 14,000 rpm (12,000 g) for 30 min and resuspended in 300 µl of diethyl pyrocarbonate (DEPC)-treated water. The RNA was then precipitated again with 1/10 volumes of 3 M sodium acetate (pH 5.2) and isopropanol. The precipitated RNA was rinsed with 70% ethanol (diluted in DEPC-treated water) and dissolved in an appropriate volume of DEPC-treated water (30–40 µl). The yield and purity of the RNA were assessed with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and the RNA integrity was determined as the RNA integrity number (RIN) with a Bioanalyzer 2100 (Agilent Technology, Santa Clara, CA, USA). A PCR-based cDNA library was constructed with the TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions.

2.2. Sequencing and de novo assembly

The transcriptome was sequenced with the Illumina HiSeq 2000 Sequencing System by Theragen (Suwon, South Korea). A total of

Table 2
Summary of BUSCO and blastp results. Transcriptome completeness was assessed with BUSCO. Species distribution of top blastp search results against the *nr* database is listed in the last column. Only top 5 species are shown for brevity with the percentage of CDSs for each species.

Dataset	The number of CDSs	Transcriptome completeness (%)	Top 5 species from blastp (%)
CDSs homologous to coral sequences	36,266	96.4	*A. digitifera* (30.76) *O. faveolata* (15.5) *S. pistillata* (15.41) *A. queenslandica* (7.44) *S. microadriaticum* (3.42)
CDSs homologous to coral but not to *Symbiodinium* sequences	28,414	75.2	*A. digitifera* (36.97) *O. faveolata* (17.7) *S. pistillata* (17.54) *A. queenslandica* (6.97) *Exaiptasia pallida* (3.84)
CDSs homologous to *Symbiodinium* sequences	51,881	75.2	*S. microadriaticum* (65.21) *A. queenslandica* (1.82) *A. digitifera* (1.29) *S. pistillata* (1.22) *O. faveolata* (1.19)
CDSs homologous to *Symbiodinium* but not to coral sequences	44,029	58.4	*S. microadriaticum* (74.02) *Chrysochromulina sp. CCMP291* (0.64) *A. queenslandica* (0.52) *Aureococcus anophagefferens* (0.3) *Ectocarpus siliculosus* (0.26)

31,921,488 read pairs (100 bp) in the FASTQ format were produced and the short-read dataset has been deposited in GenBank under BioProject ID PRJNA436760. To remove sequencing artifacts and improve the quality of the *de novo* assembly, adapters and poor-quality bases in the raw reads were trimmed with Trimmomatic v0.36 (Bolger et al., 2014), with the default parameters. This procedure extracted 5,949,582,694 bp from 30,449,429 read pairs (average length = 97.7 bp). *De novo* assembly was performed with Trinity v2.3.2 (Grabherr et al., 2011), and 347,748 contigs were generated (≥200 bp; N50 = 1335 bp; maximum length = 41,520 bp). Contigs, including candidate coding regions, were identified with TransDecoder v4.1.0 (Haas & Papanicolaou, 2016), and highly similar sequences (with a global sequence identity threshold of 95%) were clustered with cdhit-est v4.6.7 (Li & Godzik, 2006). This process identified 108,636 coding sequences (CDSs). MIxS description is available in Table 1.

2.3. Functional annotation

Our draft assembly is mixture of transcripts from the coral and symbiotic microbes including *Symbiodinium*. To estimate how many coral host and *Symbiodinium* genes are represented in the holobiont transcriptome assembly, we performed the homology search of 108,636 CDSs against other corals and *Symbiodinium* sequences. Draft genomic scaffolds and CDSs of two corals (*Acropora digitifera* and *Stylophora pistillata*) and three *Symbiodinium* species (*Symbiodinium kawagutii*, *Symbiodinium microadriaticum*, *Symbiodinium minutum*) were used as the target databases (Shinzato et al., 2011; Aranda et al., 2016; Lin et al., 2015; Shoguchi et al., 2013; Voolstra et al., 2017) and blastn search (e-value cutoff: 10^{-4}) was conducted against coral and *Symbiodinium* databases separately (Altschul et al., 1990). 36,266 (33.38%) and 51,881 (47.76%) of 108,636 predicted CDSs were aligned significantly to other coral and *Symbiodinium* sequences, respectively. 28,414 (26.16%) and 44,029 (40.53%) CDSs were aligned only one of coral or *Symbiodinium* databases, respectively, which represent the genuine *A. japonica* and *Symbiodinium* sequences. 7852 (7.23%) CDSs had strong homology to both coral and *Symbiodinium* sequences, which may indicate highly conserved genes in both corals and Symbiodiniums. However, further investigation is required to identify whether these sequences are from the host or the symbiont. Some genes may be evolved only in *A. japonica* holobiont distinctively from above listed corals and Symbiodiniums, and therefore could be missed during the blast search. If we do not consider these lineage-specific genes of *A. japonica* holobiont, 28,414~36,266 and 44,029~51,881 would be the number of CDSs for *A. japonica* and residing *Symbiodinium* in our transcriptome, respectively. These numbers are comparable to those of gene models from the genome sequencing of other species (26,275 for *A. digitifera*; 25,769 for *S. pistillata*; 36,850 for *S. kawagutii*; 49,109 for *S. microadriaticum*; 47,014 for *S. minutum*).

The quality of the *de novo* assembly and gene prediction was evaluated quantitatively based on single-copy genes that are considered to occur in most organisms. We used BUSCO v3.0.2 (Waterhouse et al., 2017) to assess the completeness of the reference transcriptome assembly and annotation of *A. japonica* holobiont. Of 303 eukaryotic benchmark genes analyzed, 299 were found in the reference transcriptome (98.7% completeness). We also separately assessed the completeness of coral and *Symbiodinium* CDSs. 75.2–96.4% and 58.4–75.2% of eukaryotic benchmark genes were represented among the coral host or *Symbiodinium* CDSs of *A. japonica* holobiont transcriptome, respectively (Table 2).

Putative genes in *A. japonica* holobiont transcriptome were annotated with blastp v2.6.0 (Altschul et al., 1990), with the NCBI non-redundant protein sequences (*nr*) as the target database. Of the 108,636 sequences, 90,014 (82.86%) had at least one hit in *nr* (e-value ≤10^{-4}). The genes most similar to coral CDSs were from other stony corals (*A. digitifera*, *Orbicella faveolata*, *S. pistillata*), while the majority of *Symbiodinium* CDSs were homologous to *S. microadriaticum* (Table 2).

Gene Ontology (GO) terms were annotated to each coding sequence using above blastp results against *nr* database, the 'gene2go' file (NCBI), the 'gene2accession' file (NCBI), and in-house scripts. 50,025 of 108,636 sequences (46.05%) were mapped to at least one GO terms. Overall distribution of top 20 frequent GO terms are displayed in Fig. 1, Supplementary Fig. 1, and Supplementary Fig. 2 for three GO main categories.

The high quality transcriptomic data generated in this study will allow us to investigate the physiological responses of *A. japonica* and its symbionts to marine environmental changes, such as increases in seawater temperature and ocean acidification.

Author contributions

SW and SY conducted sampling and RNA extraction of coral. TR and WC performed computational analysis. TR, SW, and SY wrote the paper.

Data availability

RNA-Seq reads have been deposited in GenBank under BioProject ID PRJNA436760. The transcriptome assembly has been deposited at DDBJ/EMBL/GenBank under the accession GGJR00000000 in the FASTA format. The assembly version described here is the 1st version (GGJR01000000).

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