Identification of functional genes in deep-sea corals from seamounts in West Pacific by *de novo* RNA sequencing

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Abstract

This is a study about investigation the biodiversity of the deep-sea seamount in the West Pacific and discovering the marine biological genetic resources. Coral samples were obtained using epibenthic sledge(EBS) which was one of the collection equipment of Onnuri Research Vessel in Korea Institute of Ocean Science and Technology. To classify the coral species, DNA sequences of CO1 and MSH1 were used together with morphological classification method. RNA was extracted by following the method optimized for the soft coral and de novo RNA sequencing (RNA-seq) was carried out to explore the functional genes in three deep-sea coral species, *Iridogoria splendens*, *Chrysogoria chryseis* and *Calyptrophora wyvillei*. Approximately 193,579 unigenes from *Iridogoria splendens*, 235,513 unigenes from *Chrysogoria chryseis* and 193,796 unigenes from *Calyptrophora wyvillei* were identified and those unigenes were classified their functions using NCBI Nucleotide, Pfam, Gene ontology (GO), EggNOG, and UniProt databases. This study focused on the identification of functional genes in deep sea corals and this genetic information will be used for the various analyses of secure genetic sources of target species and understand their environmental characteristics.

Introduction

A seamount is a large geologic landform that rises from the ocean floor but that does not reach to the water's surface (sea level), and thus is not an island, islet or cliff-rock. Seamounts are typically formed from extinct volcanose that rise abruptly and are usually found rising from the seafloor to 1,000–4,000 m (3,300–13,100 ft) in height. They are defined by oceanographers as independent features that rise to at least 1,000 m (3,281 ft) above the seafloor, characteristically of conical form. The peaks are often found hundreds to thousands of meters below the surface, and are therefore considered to be within the deep sea. The studies about deep sea area and animals around seamounts have been reported robustly for the last 5 years but still genetic information of deep sea animals is uncovered much. We investigated deep sea coral species specially around seamounts in this study and purposed to obtain the functional genetic information from the deep sea coral species.

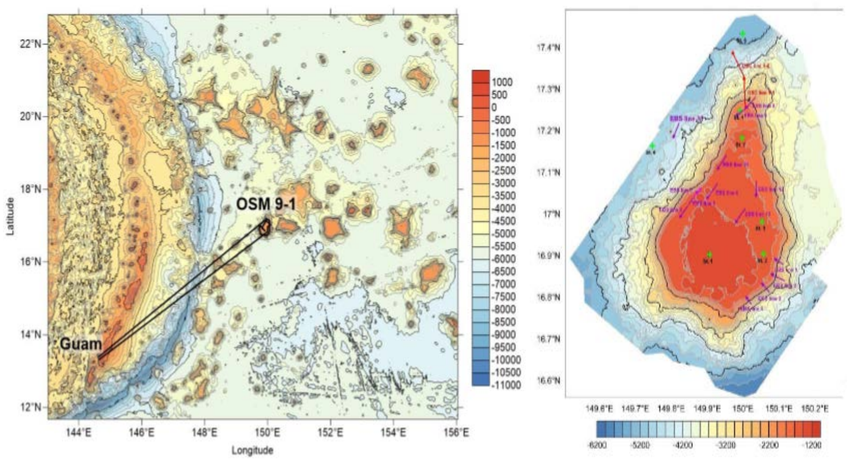

Figure 1. Sampling locations. OSM 9-1; Longitude: 149 54□ E ~ 150□ 12□ E, Latitude: 16□ 54□ N ~ 17□ 27'N

Materials and Methods

Samples were obtained using Epibenthic sledge(EBS), the collection equipment of Onnuri Research Ship. Iridogoria sp. was collected in EBS line 6. The starting latitude is 149°56.302 17°05.021 and the end point is 149°53.665 17°02.218. Chrysogoria sp. and California sp. were collected from EBS line 7.
The starting line is 149°51.483 17°02.114 and the end of the line is 149°53.074 17°03.934 latitude. After collection, only coral polyps were collected then directly frozen in the liquid nitrogen and conserved under the -80˚C until the RNA extraction. Total RNA was extracted by following the method optimized for the soft coral (Woo et al., 2005). We used HiSeq 3000 sequencing system (Illumina) and briefly we prepared the RNA for transcriptome, then pair-end sequencing and analysed with *de novo* assembly.

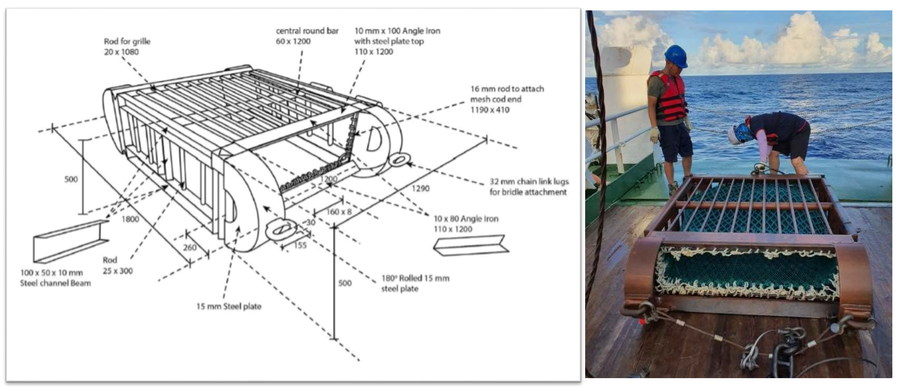

Figure 2. Detailed construction plan of the sledge (Clark and Stewart, 2016)

Results

NCBI Nucleotide, Pfam, Gene ontology (GO), EggNOG, and UniProt were used to search for Unigene's functions. *Iridogoria splendens* 26.9% of the total assembly results were function-checked, and 52,176 of the total 193,579 unigenes were found in at least one database. *Chrysogoria chryseis* 31.7% of the total assembly results were function-checked and 74,772 of the total 235,513 unigenes were found in at least one database. *Calyptrophora wyvillei* 26.4% of the total assembly results were function-checked, and 51,334 out of a total of 193,796 unigenes were found in at least one database.

Figure 3. The samples using Epibenthic sledge(EBS). A: Iridogoria splendens, B: Chrysogoria chryseis, C: Calyptrophora wyvillei

Trimming Data Stats

Index	Sample id	Total read bases	Total reads	GC(%)	Q20(%)	Q30(%)
1	2019EBS-3	14,161,503,196	140,891,522	41.44	98.78	95.69
2	2019EBS-7	11,778,930,199	118,569,498	42.84	98.55	95.25
3	2019EBS-9	11,374,025,548	113,305,728	43.14	98.71	95.55

Annotation result of various databases

Assembly	2019EBS-3	2019EBS-7	2019EBS-9
Total Unigene	193,579	235,513	193,796
GO	27,205 (14.05%)	40,596 (17.24%)	26,312 (13.58%)
UniProt	20,865 (10.78%)	31,373 (13.32%)	20,532 (10.59%)
NR	44,396 (22.93%)	66,369 (28.18%)	43,508 (22.45%)
Pfam	25,963 (13.41%)	37,870 (16.08%)	25,052 (12.93%)
EggNOG	37,872 (19.56%)	57,031 (24.22%)	37,085 (19.14%)
NT	3,586 (1.85%)	5,536 (2.35%)	3,492 (1.8%)
KO_EUK	49,920 (25.79%)	72,116 (30.62%)	49,499 (25.54%)
Overall	52,176 (26.95%)	74,772 (31.75%)	51,334 (26.49%)

Acknowledgement

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