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Massively parallel sequencing-based survey of eukaryotic community structures in Hiroshima Bay and Ishigaki Island

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ABSTRACT

In this study, we compared the eukaryote biodiversity between Hiroshima Bay and Ishigaki Island in Japanese coastal waters by using the massively parallel sequencing (MPS)-based technique to collect preliminary data. The relative abundance of Alveolata was highest in both localities, and the second highest groups were Stramenopiles, Opisthokonta, or Hacrobia, which varied depending on the samples considered. For microalgal phyla, the relative abundance of operational taxonomic units (OTUs) and the number of MPS were highest for Dinophyceae in both localities, followed by Bacillariophyceae in Hiroshima Bay, and by Bacillariophyceae or Chlorophyceae in Ishigaki Island. The number of detected OTUs in Hiroshima Bay and Ishigaki Island was 645 and 791, respectively, and 15.3% and 12.5% of the OTUs were common between the two localities. In the non-metric multidimensional scaling analysis, the samples from the two localities were plotted in different positions. In the dendrogram developed using similarity indices, the samples were clustered into different nodes based on localities with high multiscale bootstrap values, reflecting geographic differences in biodiversity. Thus, we succeeded in demonstrating biodiversity differences between the two localities, although the read numbers of the MPSs were not high enough. The corresponding analysis showed a clear seasonal change in the biodiversity of Hiroshima Bay but it was not clear in Ishigaki Island. Thus, the MPS-based technique shows a great advantage of high performance by detecting several hundreds of OTUs from a single sample, strongly suggesting the effectiveness to apply this technique to routine monitoring programs.

1. Introduction

Eukaryotic communities are usually dominated by phototrophic and heterotrophic protists; in coastal environments, dinoflagellates and diatoms predominate, although micro-metazoans belonging to a variety of animal phyla can also form a significant component of the community (Manabe et al., 1994; Nishikawa et al., 2010). Biodiversity studies of aquatic microbial eukaryotes require the identification and enumeration of organisms with an extremely wide taxonomic diversity. Historically, protist diversity studies have largely relied on morphologically based identification by microscopic observation (Tomas, 1997). Since most protist species have been defined morphologically, identification of protists in environmental samples is particularly difficult since some species within a genus lack distinctive morphological characteristics, especially for micro-eukaryotes that are smaller than 10 μm in body length. For this reason, cultivation-independent molecular surveys have been applied through cloning and sequencing after polymerase chain reaction (PCR), which have demonstrated that protist diversity has been underestimated by orders of magnitude. These findings have thus stimulated renewed interest in protist diversity research within the last decade (Diez et al., 2001; López-Garcia et al., 2001; Moreira and López-Garcia, 2002; Moon-van der Staay et al., 2006).

The increase in the numbers of sequences registered in genetic databases for a wide range of microbial eukaryotes offers the possibility to greatly improve the technology used for the study of biodiversity, community structure, and adaptation, and to understand the evolutionary relationships among the many protistan lineages (Caron et al., 2009). Genetic approaches have also been extensively used to assess the composition of natural assemblages of protists from a variety of marine ecosystems such as polar regions (Darling et al., 2000; Luo et al., 2009; Majaneva et al., 2012), the deep sea (López-Garcia et al., 2001), the ocean surface (Fuller et al., 2006; Shi et al., 2011), anoxic waters (Alexander et al., 2009; Stock et al., 2009; Behnke et al., 2010), and coastal environments (Massana et al., 2004; Medlin et al., 2006; McDonald et al., 2007).

Abbreviations: MPS, massively parallel sequencing; NMDS, non-metric multidimensional scaling; OTUs, operational taxonomic units.

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Roche 454 pyrosequencing has become a popular method for conducting studies on protistan diversity and species richness in marine and freshwater ecosystems owing to the recent development of next-generation sequencing (NGS) technologies. Although this new technology delivers high-throughput performance and allows detection of several thousands of operational taxonomic units (OTUs) from marine and freshwater ecosystems (Cheung et al., 2010; Medinger et al., 2010; Nolte et al., 2010; Edgcomb et al., 2011; Monchy et al., 2012), several problematic issues remain, i.e., 1) formation of PCR-generated artifacts such as chimeric sequences developed during PCR amplification (Qiu et al., 2001; Haas et al., 2011; Quince et al., 2011; Edgar et al., 2011); 2) NGS artifacts such as homopolymer errors introduced during the NGS process (Huse et al., 2007, 2010; Kunin et al., 2010; Schloss et al., 2011); and 3) impacts of identification accuracy on taxonomic assignment and comparison of biodiversity (Bazinet and Cummings, 2012; Tanabe and Toju, 2013).

Hiroshima Bay is located in the western part of Japan, and its surface area is about 1000 km^2, with a mean depth of 25 m. There are many small islands in Hiroshima Bay, which impede the exchange of seawater in the bay. Therefore, the water conditions of the bay are affected by meteorological events of various time scales as well as by local human activities. The seawater and bottom sediments in the closed area of the northern part of Hiroshima Bay are extremely polluted by organic substances, and many kinds of red tides have occurred in this region in the summer (Kimura et al., 1973). The climate is temperate, and the mean monthly seawater temperature is highest in August (30.8 °C) and lowest in March (8.4 °C), according to observations conducted in Hiroshima City from 1971 to 2011 (Hiroshima Prefectural Technology Research Institute, http://www2.ocn.ne.jp/~hfes/senkai.html). The average concentrations of dissolved inorganic nitrogen DIN (NH_4-N, NO_2-N + NO_3-N), PO_4-P, and chlorophyll-a in the island varied in the ranges of 0.4–8.8 μM, 0.08–0.67 μM, and 4.7–21.7 μg L^{-1}, respectively, in the study periods (Kamiyama et al., 2005), clearly showing that the waters are eutrophic.

Ishigaki Island is located in the Ryukyu Archipelago (an area of approximately 229 km^2) in the southernmost region of Japan, and fringing reefs are developed around the island in general (Fig. 1). The climate is subtropical, and the mean monthly seawater temperature is highest in July (29.8 °C) and lowest in January (21.3 °C), according to observations conducted at Ishigaki Island from 2002 to 2005 (Japan Oceanographic Data Center, http://www.jodc.go.jp/index_j.html). Average concentrations of NH_4-N, NO_2-N + NO_3-N, PO_4-P, and chlorophyll-a in the island varied in the ranges of <0.2 μM, <0.4 μM, 0.016–0.31 μM, and 0.05–0.20 μg L^{-1}, respectively, in the study periods (Abe, 2007; Morimoto et al., 2010), clearly showing that its waters are oligotrophic. Clear seasonal changes in biological productivity have been observed in the reefs at Ishigaki Island (Kayanne et al., 2005a). Although the East China Sea (ECS) water does not directly affect the reefs because of the Kuroshio Current that usually flows between Ishigaki Island and the ECS, DIN content is often relatively rich in the oceanic waters around the reefs (Miyajima and Hata, 2007).

In this study, we compared the eukaryote biodiversity between Hiroshima Bay (as a eutrophic temperate water system) and Ishigaki Island (as an oligotrophic sub-tropical water system) in Japanese coastal waters by using the massively parallel sequencing (MPS)-based technique as a preliminary study, and discuss the potential of this technology for introducing larger scale monitoring surveys of eukaryotic diversity.

2. Materials and methods

2.1. Sampling and DNA extraction

Seawater samples were collected monthly from the surface layer with a plastic bucket from June to October in 2009 in Hiroshima Bay (132° 15′ E, 34° 16′ N) and Ishigaki Island, Japan (124° 13'E, 24° 27′ N) (Fig. 1). To trap all the plankton in the seawater samples, 1000 mL of the seawater was filtered through 8-μm pore-size polycarbonate filters (Nuclepore membrane; GE Healthcare; Tokyo, Japan), and the seawater that passed through the 8-μm filters was further filtered through 1-μm pore-size filters (GE Healthcare). The filters were stored in a deep freezer (−80 °C) until use. For DNA extraction, a 5% Chelex® suspension (Chelex 100 Molecular Biology Grade Resin; Bio-Rad Laboratories Inc.; Richmond, CA) was prepared by dispersing the resin into ultra-pure quality water. For effective DNA extraction from plankton components trapped on the filters, the filters were cut in half, placed in 1.5-mL tubes (A.150; Assist; Tokyo, Japan), and 150 μL of 5% Chelex buffer was added. The plankton cells were crushed using a pellet pestle motor (Kontes Glass; Vineland, NJ, USA) for 60 s, and 350 μL of buffer was added to make up a final volume of 500 μL. DNA was extracted by heating the 1.5-mL tubes at 95 °C for 20 min (Nagai et al., 2012). DNA extracted from the 8-μm filter and the 1-μm filter was mixed equally (50 μL + 50 μL) and used as template DNA.

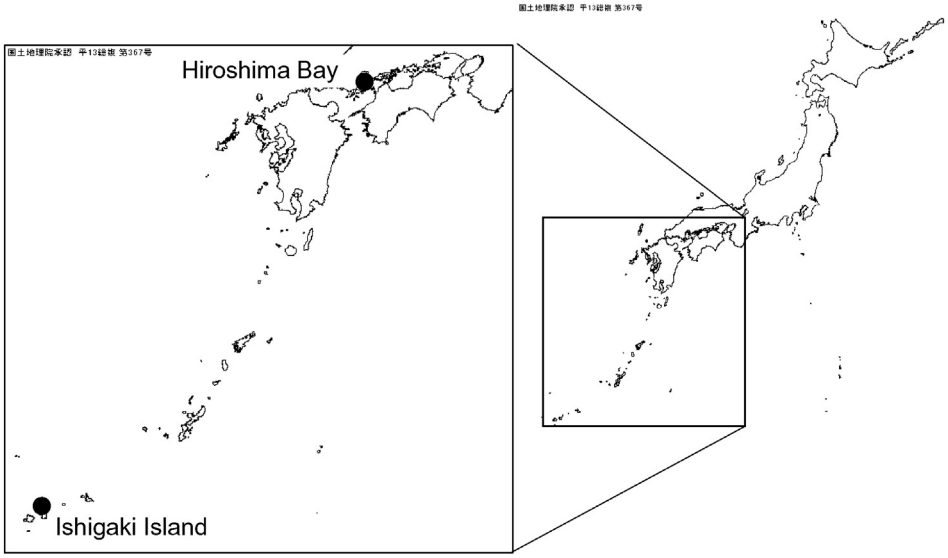

Fig. 1. Sampling locations in Hiroshima Bay and Ishigaki Island, Japan.

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