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Marine fungal communities in water and surface sediment of a sea cucumber farming system: habitat-differentiated distribution and nutrients driving succession

Xiaohong GUO[a,b], Qianqian ZHANG[a], Xiaoli ZHANG[a], Jiasong ZHANG[c], Jun GONG[a,*]

[a]Laboratory of Microbial Ecology and Matter Cycles, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
[b]University of Chinese Academy of Sciences, Beijing 100049, China
[c]South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China

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ABSTRACT

The planktonic and benthic fungi in a sea cucumber farming system were simultaneously investigated on three sampling dates. Analyses of SSU rRNA gene libraries of four samples revealed 131 fungal operational taxonomic units, of which 58 % were potentially novel. Chytridiomycota, Blastocladiomycota and Monoblepharidomycota were detected only from sediment, whereas ascomycetes and basidiomycetes dominated in sediment and water, respectively. Cryptomycota phylotypes were detected in both water and sediment samples. Based on terminal-restriction fragment length polymorphisms, distinct succession and contrasting community structure were found between planktonic and benthic habitats. Redundancy analysis indicated that the concentration of dissolved silicate in surface water and N:P in porewater were the most significant abiotic variables shaping the planktonic and benthic communities, respectively. This study indicates that plankton and benthos are distinct habitats for fungal distribution even in shallow coastal systems, and nutrients and stoichiometric ratios play important roles in driving succession of fungal communities.

Introduction

Marine fungi act as decomposers of complex organic matter in the water column and sediment, colonists of drifting wood, and symbionts or parasites of phytoplankton, plants and animals, and thus play a vital role in the functioning of coastal ecosystems (Hyde, 2002; Raghukumar, 2008; Wang and Johnson, 2009; Jobard et al., 2010; Gleason et al., 2011; Jones and Pang, 2012). While our knowledge of the diversity of culturable marine fungi has been furthered by morphology-based surveys (e.g., Raveendran and Manimohan, 2007; Jones et al., 2009; Pang et al., 2010; Velez et al., 2013), the recent application of culture-independent techniques has advanced our understanding of the diversity

* Corresponding author.
E-mail address: jgong@yic.ac.cn (J. Gong).
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and ecology of fungi in a range of coastal habitats (Pang and Mitchell, 2005; Richards et al., 2012; Manohar and Raghukumar, 2013). By using rDNA-based community profiling methods, library construction and sequencing, novel fungal lineages and phylotypes and specific community compositions have been revealed in association with brown algae (Zuccaro et al., 2004), sponges (Gao et al., 2008) and coral (Amend et al., 2012), in the water column of the Hawaiian coast (Gao et al., 2010) and oxygen-depleted regions of the Arabian Sea (Jebaraj et al., 2010). A new fungal phylum, the Cryptomycota, has been proposed for some of the lower fungi frequently detected in environmental samples (Jones et al., 2011). Rich fungal populations in anoxic mangrove sediments have been detected (Arfi et al., 2012) and the distribution patterns of fungi in either the water column or sediments have also been studied (e.g., Gao et al., 2010; Mohamed and Martiny, 2011). Nevertheless, little is known about the distinctive communities of fungi in water and sediment within a given aquatic ecosystem.

Coastal ecosystems receiving massive terrestrial nutrients and organic matter from land are facing increasingly intensive pressures from human activities and climate changes. One of the most intensive human activities affecting coastal zones is land based and off shore aquaculture, of which China has the largest output in the world. Microbes have been a focus for research in aquaculture systems because of their roles in nutrient cycling, water quality, disease control and environmental impact of the resulting effluent (Moriarty, 1997). However, fungi in aquaculture systems have rarely been investigated using molecular approaches, and the mechanisms controlling the fungal communities of these systems remain poorly understood.

In this study, we have documented, for the first time, the molecular diversity and community composition of fungi in a mariculture environment by taking the semi-intensive aquaculture system as a model. We hypothesized that: (1) the fungal communities in the coastal aquaculture environment were habitat-related, with community differences between water and sediment, and between farming and non-farming ponds; and (2) different environmental factors regulated the dynamics of fungal communities in water and sediment.

Materials and methods

Study sites and sampling

The sea cucumber farming system, located in Yantai, Shandong Province, northern China (37°26′44″N, 121°32′4″E), consisted of two ponds: a farming pond (FP) where the holothurians were farmed, and a supply pond (SP) which had a consistent volume of seawater but no cultured organisms throughout the year. Both ponds are elongate in outline, with a distance of about 1 km to the coastline. These two ponds were connected with a 50 m long canal, through which the FP could receive "raw" water from the SP under management. The SP is larger than the FP, with an area of about 30 and 18 km^2, respectively (Fig 1). Both ponds had been used for sea cucumber farming for about 5 yr, with water depths around 1.2 m and sandy sediment.

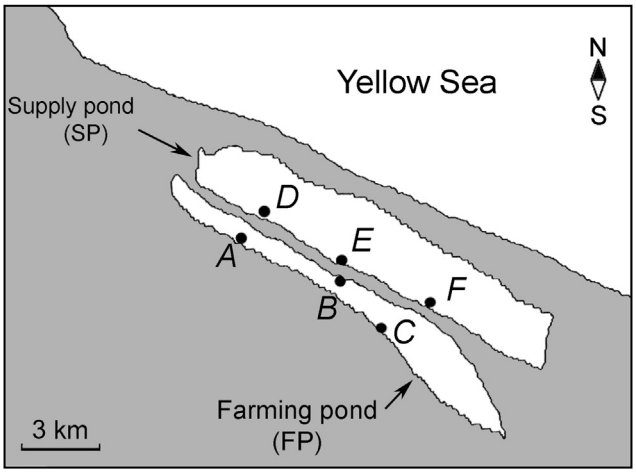

Fig 1 – Location of study sites in a sea cucumber farming pond (A–C) and a supply pond (D–F) of an aquaculture system on the coast of Yantai.

A total of six sites (A–C and D–F in the sea cucumber farming pond and the supplying pond, respectively) were sampled on three dates: 7 Oct. and 5 Nov. 2012, and 10 Jan. 2013, corresponding to the stages at which the sea cucumbers were fed with artificial diets, and the non-feeding, and wintering periods, respectively (Fig 1). At each site, about 2 l of surface water was pre-filtered through a 20 μm mesh to remove large plankton and particles, and subsequently filtered onto a 0.2 μm pore membrane (PALL, USA) under vacuum. The membrane was immediately put into a sterile tube. Sediment was collected by coring, and the surface layers (top 2 cm) were put into a sterile plastic bag. The collected samples were stored at −80 °C until DNA extraction. Filtered water was maintained at 4 °C and brought back to the laboratory for chemical analysis. We were unable to obtain samples from sites C and F on the third sampling date due to accessibility issues. Thus, a total of 32 water and sediment samples, thereafter referred to as A1-W, A1-S (the water and sediment samples collected in the first sampling at site A) and so on, were investigated and analyzed (see Table 1).

Determination of environmental variables

Water temperature (Temp), salinity (Sal), concentrations of dissolved oxygen (DO) and chlorophyll *a* (Chl-*a*) in surface waters were measured on site with an electronic probe (Hydrolab MS5, Hach, USA). Porewater in sediments was extracted by centrifugation and total organic carbon (TOC) was measured with the Shimadu TOC VCPH. Dissolved inorganic nitrogen species (NH_4^+, NO_3^- and NO_2^-), dissolved silicate (DSi) and soluble reactive phosphorus (PO_4^{3-}) were determined by autoanalyser III (Seal, Germany).

DNA extraction and PCR amplification

The environmental DNA in the sediment and water samples was extracted using the Fast DNASpin Kit for soil (MP Biomedicals, Irvine, CA, USA) following the manufacturer's