



## Research papers

# Archaeal community structure and response to ark shell bioturbation in typical intertidal mudflats, Southeast coast of China



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## ABSTRACT

Although many studies have investigated archaeal distribution in various environments, studies regarding the archaeal community structure in intertidal mudflat sediments in coastal areas are not enough. In addition, the response of archaea to bivalves bioturbation has not been reported before, nevertheless, bioturbation effects on microbes are key to many aspects of ecosystem function. In this paper, clone library construction, Restriction Fragment Length Polymorphism (RFLP) and Denaturing Gradient Gel Electrophoresis DGGE (DGGE) were used to study the archaeal community structure in two typical intertidal mudflats in Southeast coast of China: the one mainly used for aquaculture in a semi-enclosed Bay (Sansha Bay), and the other one mainly used for recreation in a developed city (Xiamen). In addition, the potential influence of sampling time and the in situ bioremediation by artificially introducing bivalve ark shell (*Tegillarca granosa*) to mudflat sediments on archaeal communities were also investigated. A total of 283 clones were randomly selected from four clone libraries and were divided into 42 RFLP patterns. All the cloned sequences fell into three phyla: the Thaumarchaeota (group MGI), Crenarchaeota (group MCG) and Euryarchaeota (groups MBG-D, MBG-E, VAL III and DHVE 6), in which Thaumarchaeota MGI was dominant, occupying 75.6% of total clones. Crenarchaeota MCG and Euryarchaeota accounted for 15.2% and 9.2% respectively. The archaeal diversity in Xiamen intertidal sediment was lower than that of Sansha Bay. Introducing ark shell seemed to increase archaeal diversity and the proportion of MCG and decrease the proportion of MGI in the clone library. DGGE profiles showed that most of the archaea populations were metabolically active, and the fingerprint clustering results were basically consistent with the results from clone libraries.

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## 1. Introduction

The intertidal zone, located at the junction of sea and land, is a simple ecosystem. It is narrow but has high productivity and creates great economic values. It is the important habitats for many organisms, and provides food and resources for people (Ortega-Morales et al., 2010). However, with the rapid development of marine industries and local economy, the coastal ecosystem is seriously polluted, and the deterioration in intertidal zones requiring global attention and action.

Shellfishes are filter feeding bivalves which can affect the distribution of organics by filtering seawater, defecating, and transforming particles into dissolved organic matters or biological substances through metabolism activities (Dame et al., 1984). The biological filtration and sedimentation of shellfish can alleviate the

nutrient load of eutrophicated coastal area (Qing et al., 2001) and thus has good prospects in environmental remediation (Chen et al., 2012). Ark shell (*Tegillarca granosa*) is a typical filter feeding organism and a traditional Chinese aquaculture shellfish. Many studies have reported the influence of filter feeding bivalves on nutrients in sediments (Welsh et al., 2015; Sun et al., 1999), but studies regarding their influence on environmental microbes are not enough. Microbes are very sensitive to environmental changes and can be used as indicators for assessing and predicting environmental health (Lin et al., 2008). Currently several studies reported the dynamics of microbial community structure during environmental remediation process, but the involved microbes were mainly bacteria (Fabiano et al., 2003; Simarroa et al., 2013). There is little information about the dynamics of archaeal communities during the environmental remediation process.

Archaea are divided into five recognized phyla: Euryarchaeota, Crenarchaeota Thaumarchaeota, Korarchaeota and Nanoarchaeota. They were previously thought to be present only in extreme

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environments, but since the first discovery of archaea in marine-oxygenated water column (DeLong, 1992; Fuhrman et al., 1992), many studies have shown that archaea are abundant and widespread in common habitats, such as ocean waters and sediments, freshwater sediments, lakes, and soils (DeLong, 1992; Fuhrman et al., 1992; Schleper et al., 2005; Auguet et al., 2010). It was reported that archaea account for one-third of the prokaryotes in the ocean, and Crenarchaeota was the most abundant phylum (Karner et al., 2001). In tidal flat sediment, archaeal community is remarkably diverse (Kim et al., 2005) and archaea genes account for 1.6–4.8% of the metagenomic gene libraries (Kim et al., 2009). Compared with bacteria, archaea have unique physiological processes and ecological functions. For instance, the methanogenic archaea populations, commonly found in wetland, are capable of anaerobically decomposing organic matter and producing methane as a metabolic byproduct. This anaerobic mineralization of organics plays an indispensable role in anaerobic wastewater treatments (Tabatabaei et al., 2010). In addition, previous studies (Leininger et al., 2006) have shown that the *amoA* gene (encoding a subunit of the key enzyme ammonia monooxygenase) copies of archaea were far more abundant than bacterial *amoA* genes. Ammonia-oxidizing archaea (AOA) may be the most abundant ammonia oxidizers in soil ecosystems on Earth and play a more important role than ammonia-oxidizing bacteria (AOB) in marine nitrogen cycling (Kalanetra et al., 2009). Thus, studies on structures of archaeal community and their distribution patterns are essential to understand their roles in regulating biogeochemical process.

Archaea are difficult to cultivate (Prokofeva et al., 2000; Peralova et al., 2005) and currently only a minority of pure cultures was obtained. The development of culture-independent molecular techniques provides convenience for studying archaea. Using molecular techniques, archaea in various environments, including different depth of sea (Hu et al., 2011), freshwater lake sediment (Koizumi et al., 2004), high Arctic peat (Høj et al., 2008), soil (Tourna et al., 2008), fermented seafood (Roh et al., 2009) and so on, have been studied. However, studies regarding the archaeal community in intertidal mudflats and their changes in some special biogeochemical process are not enough. Two recently published papers reported the influence of polychaetes bioturbation on archaeal community in oil-contaminated mudflat sediments (Stauffer et al., 2014) and the impacts of burrowing mud shrimp on temporal variation in bacterial and archaeal nitrogen-cycling gene abundance in coastal sediments (Laverock et al., 2014), respectively. Nevertheless, the archaeal community response to bioremediation by artificially introducing bivalves to tidal mudflat has not been reported before.

In the present study, two typical intertidal mudflats were chosen for studying the archaea community structure in southeast coast of China by 16S rDNA clone library construction, Restriction Fragment Length Polymorphism (RFLP), Denaturing Gradient Gel Electrophoresis (DGGE) and sequences analysis. In addition, the potential influence of sampling time and the in situ bioremediation by artificially introducing ark shell (*Tegillarca granosa*) to intertidal mudflats on archaeal community structure were also involved, hoping that the information present here will help to deepen the understanding of archaeal community structure in intertidal mudflats, and provide a foundation for further study of archaeal functions in macrobenthic bioremediation processes.

## 2. Materials and methods

### 2.1. Study area

The study area was located in two kinds of typical intertidal

mudflats in Fujian, southeast coast of China: (1) Sansha Bay, representing an aquaculturing mudflat, and (2) Xiamen, representing a recreational mudflat that is adjacent to developed city, Xiamen, respectively. Sansha Bay is located in the northeast of Fujian Province and is a typical enclosed or semi-enclosed bay. It is a natural harbor of China, and had good water quality in history. It also is the spawning and breeding grounds for the famous large yellow croaker of China and a variety of economic animals. With the rapid development of aquaculture in recent years, Sansha Bay became the largest marine cage breeding base in China. Due to the deposition of residual baits, feces, plant and animal remains, the eutrophication is increasingly serious. Furthermore, the weak ability of self-purification in inshore enclosed bay undoubtedly worsens the already grave environment problems. It is reported that the seawater was under serious eutrophication and the organic pollution was also very serious in Yantian Harbor and Lumen Harbor of Sansha Bay (Hu et al., 2014). In the present study, the bivalve ark shells were used as restorers for repairing the contaminated mudflats in north of Yantian Harbor, and the response of Archaea to the bioremediation was studied in this study.

Xiamen coastal tideland represents another kind of intertidal environment. Xiamen is an important central city in southeast coast of China, it has dense population and advanced economy. The coastal environment pollution in Xiamen was mainly derived from industrial and domestic sewage discharge and aquaculture self pollution. However, aquaculture has been exited from Xiamen coastal area since 2002 for protection of coastal environment, and the urban and domestic sewage treatment rate has now reached up to 93% (The datum comes from Xiamen's local government website, [http://hbcm.xm.gov.cn/cjcg/hjjs/201108/t20110812\\_420851.htm](http://hbcm.xm.gov.cn/cjcg/hjjs/201108/t20110812_420851.htm).) in Xiamen.

### 2.2. Field sampling

The bioremediation test area in Sansha Bay covered about 15 acres, with longitude ranging from 119°47'49" to 119°47'53", and latitude ranging from 26°50'28" to 26°50'32". The bivalve ark shell (*Tegillarca granosa*) was chosen as restorer, and a total of 3300 kg ark shells were sowed in the test area at the end of April 2013. The average seeding density was 0.33 kg/m<sup>2</sup>, about 75 individuals per square meter. The nearby intertidal mudflat sediment without introducing ark shell was taken as control. The sampling site in Xiamen coast was located at Jimei district, with a latitude of 118°6'20" and a longitude of 24°34'49".

Sampling was carried out during low tide period. Four samples were taken and three of them were from Sansha Bay (prefixed with SS\_) and the other one was from Jimei, Xiamen (Prefixed with JM\_). For each sample, three subsurface sediments (each 1–2 kg) were collected at three different sites and were pooled, mixed thoroughly and about 200 g subsample was taken as one sample and was placed in a sterile disposable centrifuge tube. All samples were immediately transported to the laboratory for further analysis. The detail information for each sample was listed in Table 1.

Notes for the samples: "SS\_" indicates samples collected from Sansha Bay, "JM\_" means sample collected from Jimei, Xiamen; "Nov" and "Jun" indicate that the sampling time was in November and June respectively; "\_Teg" means the sample was collected from ark shell (*Tegillarca granosa*) introduced sediment.

### 2.3. Nutrients analyses

All samples were dried in a freeze-drier (Labconco, Kansas City, MO, USA), and the total organic carbon (TOC) was measured by a Vario Max CNS analyzer (Elementar, Hanau, Germany). The total nitrogen (TN) was determined by Kjeldahl method, and the total phosphorus (TP) was measured by ammonium molybdate spectrophotometric method.

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