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Microbiological community of the Royal Palace in Angkor Thom and Beng Mealea of Cambodia by Illumina sequencing based on 16S rRNA gene

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ABSTRACT

Angkor temples in Cambodia, an icon of Khmer civilization, display the ancient culture by bas-relief on the sandstone surface of different temples, which are being destroyed by physical, chemical and biological processes for more than a thousand years. To investigate the bio-erosion of temple sandstone at the Royal Palace of Angkor Thom and Beng Mealea in Cambodia, Next Generation Sequencing (NGS) Illumina sequencing technology based on 16S rRNA gene was performed on samples of biofilm and exfoliated sandstone materials to identify the microbial community composition. After quality filtering the raw data, 678,115 quality reads were obtained for bacterial 16S rRNA gene from a total of 13 samples with high Goods coverage and satisfactory rarefaction curves. Higher bacterial diversity was detected in exfoliated sandstone materials than the biofilms, but the lowest in the lower layers of the biofilm than the top layers. At the phylum level, 4 phyla, namely Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi, were the most common and dominant bacterial groups in these samples with each contributing to greater than 3.7% of the total abundance. Both Firmicutes and Gemmatimonadetes were the dominant phyla detected only in exfoliated materials, while Cyanobacteria, Chloroflexi, and unassigned bacteria were more abundant in the biofilms. Hierarchical cluster analysis at the genus level showed that the distribution of bacterial community composition between exfoliated materials and biofilms was significantly different. The microbiota of Beng Mealea and the Royal Palace was different, especially for the biofilm samples. The correlation of environmental factors and bacterial community structure suggested that the nitrification process was more active at Ben Mealea, which might contribute to biodeterioration. This analysis of microbiota in these biofilms and sandstone exfoliation materials provides further information on the responsible microorganisms involved in geobiochemical processes at Angkor monuments and preservation strategies under tropical climate conditions.

1. Introduction

Ancient sandstone buildings are valuable cultural heritages distributed all over the world, but severe deterioration and damage of them have resulted in an irreversible loss of them, especially the bas-relief and writing on the surfaces. Pharaonic sandstone monuments in Luxor, Egypt (Fitzner et al., 2003), ‘Yellow block’ sandstone buildings in Sydney (Friolo et al., 2003), Angkor monuments in Cambodia and surrounding Southeast Asian countries (Uchida et al., 2000; Li et al., 2008; Meng et al., 2016, 2017; Liu et al., 2018) and many other historical sites are suffering from such destruction and damage. This has been brought to a great and worldwide attention for protection and preservation of these sandstone temples/monuments and cultural heritage through further research for new techniques and strategies available.

As an icon of Khmer civilization, Angkor monuments in Cambodia and the adjacent countries are a religious shrine and historical archives of their ancient culture through bas-relief on the sandstone at different temples (Headley, 1977). Unfortunately, the exterior surfaces of the sandstone are covered by active growth of phototrophic and heterotrophic microorganisms and plants (Lan et al., 2010; Li et al., 2008, 2010; Kusumi et al., 2011, 2013; Liu et al., 2018), which alter the sandstone integrity by physical, chemical and biological processes for over almost a thousand years, resulting in the destruction of the bas-relief and loss of the cultural value under tropical climate conditions (Uchida et al., 2000; Meng et al., 2017; Liu et al., 2018). Among them,

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colonization of sandstone by microorganisms as biofilm serves several roles, including protection from physical damage initially, accelerated erosion and degradation of the sandstone because the structural material is porous with the capacity of trapping and retaining water from the plentiful rainfalls of the tropical region (Liu et al., 2018). In addition to the tropical climate conditions in Cambodia, the sandstone material provides a suitable substratum for the colonization of different types of microorganisms to form biofilms (Lan et al., 2010; Mol, 2014; Sand and Bock, 1991; Sand et al., 1991). Though nutrients available on the fresh sandstone surface are very limited, autotrophic/phototrophic microorganisms including the pioneer lichen are capable of colonizing initially through photosynthesis to immobilize atmospheric CO_2 onto the sandstone surface to provide further opportunities for heterotrophic microorganisms to establish colonization and grow. Once autotrophic microorganisms establish a biofilm community on sandstone surface, heterotrophic bacteria can then start their colonization and cause a rapid increase of the biodiversity in the biofilm community (Gu et al., 1998; Ortega-Morales et al., 2000; Scheerer et al., 2009; Gu and Mitchell, 2013). As soon as a well-defined microbial community is formed, respective biochemical activities would make a significant impact on sandstone structural integrity and damage the surface artworks (McNamara and Mitchell, 2005; Hu et al., 2011, 2013; Meng et al., 2016, 2017; Liu et al., 2018). In addition, the bacterial biofilms can spread further and occupy a greater area to cause severe damage and result in the flaking-off of fairly large and substantial pieces of the sandstone (Hirsch et al., 1995). After the initiation of the exfoliation, microorganisms grow more aggressively due to the abundantly available nutrients in the attacked area, leading to the structural failure of the structure. In the last decade, several studies were performed on the bacterial composition of the biofilm on the sandstone surface of Angkor temples in Cambodia, providing some basic information on the microflora associated with the UNESCO cultural heritage (Lan et al., 2010; Gaylarde et al., 2012; Kusumi et al., 2013; Meng et al., 2017). However, no attempt has been made to analyze the bacterial community in the exfoliation materials from sandstone for possible mechanisms of destruction.

Angkor Thom as the capital city of the Khmer Empire with great historical value attracted not only a large number of tourists of more than two million annually, but also archaeologists, architects, and biologists. Most of the Angkor temples are opened to tourists; it is a major challenge to preserve and protect the ruins in the open environment of tropical conditions (Winter 2008). The microbial biofilms and community on surfaces of these temples have been studied in the past decade to provide basic information for preservation and conservation of them (Lan et al., 2010; Li et al., 2011; Kusumi et al., 2013; Meng et al., 2017; Liu et al., 2018). However, very little is known about these temples at different locations to allow a comprehensive understanding of the current conditions and the potential damage.

To study the bioerosion of the culture heritages, it is very important to investigate and identify the microbial community composition and find out the active microorganisms responsible for altering the local environmental conditions biochemically, and further establish the relationship between the functional microbial groups and the material attack to formulate the effective restoration and maintenance stgurategies for culture heritage (Otlewska et al., 2014). For decades, culture-dependent approaches have been applied to isolate and identify the microorganisms in the community from target samples, but only 0.1–1% of the bacteria can be cultured, and then isolated and identified by the culture-dependent method by conventional microbiology (Schabereiter-Gurtner et al., 2001). More recently, molecular methods, particularly metagenomics has been developed to reveal the microbial community based on 16S rRNA gene for bacteria and archaea, and 18S rRNA or additional genes for eukaryotes (Gonzalez et al., 2003; Lan et al., 2010; Dakal and Arora, 2012). Bacteria in the biofilms formed on the stone surface were investigated by 16S rRNA gene in the Catacombs of St Callixtus in Italy (De Leo et al., 2012), Egyptian Tombs in Egypt (Abdel-Haliem et al., 2013), Roman Necropolis at Carmona in Spain (Šmerda et al., 2006) and Bayon temple of Angkor Thom in Cambodia (Lan et al., 2010). Although clone library construction, DGGE, ARDRA, and T-RFLP are among the most common molecular methods used to obtain the microbial community information and have already been applied on analysis of cultural heritage (McNamara et al., 2006; Capodicasa et al., 2010; De Felice et al., 2010), the Next-Generation Sequencing (NGS) is a newly developed method of characterizing the microbial community phylogeny and diversity faster, more accurate and economical with a deeper data recovery than those mentioned above (De Felice et al., 2010). For any investigations of microbiota on the surface of cultural heritage and the surrounding environment, where the biomass is relatively lower and the sample size is a serious concern, NGS is a prime candidate method for such analysis to obtain the data necessary with high confidence.

Beng Mealea is a major classical monument of the Angkor period and Royal Palace is the Khmer Empire King's residence. They are both important sites in the history of the Angkor City and suffered from severe deterioration and physical damage to the structure. Therefore, it is necessary to study the biofilms and exfoliation materials of them to yield a better understanding of microflora and potential biochemical processes responsible for bio-erosion of these Angkor monuments.

In this study, the composition and diversity of the bacterial community in the biofilm and exfoliated sandstone materials were sampled from the Royal Palace of Angkor Thom and Beng Mealea were analyzed using NGS Illumina sequencing based on 16S rRNA gene to gain insights of the microbial community information. In addition, ammonia, nitrite and nitrate ions in the sandstone materials were also quantified to provide information on the biochemical processes involved in the generation of nitric acid and nitrous acid which are responsible for the biodeterioration of the sandstone. Moreover, the bacteria involved in nitrite/nitrate forming processes including nitrogen fixation, ammonium oxidation and nitrite oxidation were elaborated in the current study.

2. Materials and methods

2.1. Site description and sampling

The samples used for this study were collected on August 11, 2017 at the Royal Palace of Angkor Thom and Beng Mealea, both located in the surrounding of Siem Reap in Cambodia (Fig. S1). Both sites are under typical hot and humid climate of the tropic with an average precipitation of 151 mm and the temperature between at 25 °C–32 °C in the month of August (WMO, 2016). The central tower of the Royal Palace of Angkor Thom is not accessible to visitors and selection of it for sampling also considered potential disturbance prior to sampling to avoid severe human impact.

Two types of samples were collected from each monument: exfoliated/degraded sandstone materials loosely on sandstone block and visible biofilms on the sandstone wall (Fig. 1). At the time of sampling, the top layer of loose materials was removed aseptically using sterile equipment and then fresh materials were collected and transferred into sterile plastic bags. Microbial biofilms from the surface of the structural sandstone wall were taken from areas without any visible disturbance physically and the sampling procedures followed those reported previously (Kusumi et al., 2013; Meng et al., 2017). Biofilm sampling was conducted at selected locations for multiple times on the same location to obtain samples for spatial information of the microbial community on surfaces without damage of the sandstone materials. All samples were kept in an ice-cooled box and then stored in the refrigerator for preservation at frozen condition (−20 °C) prior to transportation back to the laboratory for further analyses. They were packed with coolant during transportation from Cambodia to Hong Kong. Samples of sandstone materials were analyzed for basic physical and chemical properties and the data are presented in Table 1.