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Differences of Microbial Community on the wall paintings preserved *in situ* and *ex situ* of the Tiantishan Grottoes, China

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ABSTRACT

Tiantishan Grottoes, a famous site well known for its historical status in the spread of Buddhism art in ancient China, were selected for a comparison and analysis of microbial taxonomic characteristics on the wall paintings under different preservation conditions: *in situ* and *ex situ* conservation. A total of 12 samples were collected from three different cave wall paintings preserved *in situ* or *ex situ*. The 16/18S rRNA gene-based sequences revealed a high bacterial diversity and a relative low fungal abundance, including bacterial groups Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Cyanobacteria, Acidobacteria, Deinococcus-Thermus and Fusobacteria; and fungal groups Ascomycota and Basidiomycota. Among them, two bacterial genera of the *Promicromonospora* and *Planomicrobium* and fungal order of the Sordariales and the family of Trichocomaceae were dominant in the samples preserved *ex situ*. Some of them have been reported at other cultural heritage sites and associated with the biodeterioration of cultural relics. The over-growth of these microbes led to the abundant filaments formed visible on the surface of the *ex situ* wall paintings, which preserved under museum conditions. Application of preservation materials including animal glue and wet gypsum to take them off from grottoes and strengthen, and subsequent long-term preservation under poor conditions in museums were mainly responsible for the microbial outbreaks. To avoid similar problems in the future, reasonable intervention measures and strict micro-environmental control must be implemented to the *ex situ* preservation of wall paintings. Our results have profound significance for clarify the occurrence of microbial invasions and mechanisms on the wall paintings; it is helpful to development a reasonable artificial intervention measures for conservation work of the wall paintings in the future.

1. Introduction

Microorganisms, including bacteria, fungi, archaea and cyanobacteria, are capable of colonizing on surfaces of a wide range of materials and could result in the biodeterioration of the substratum materials, particularly for historical objects exposed to changing environmental conditions, such as temperature, relative humidity, pH and sun light (Miichaelsen et al., 2006). These organisms dwelling on wall paintings may induce aesthetic and structural damage such as pigment discoloration, contamination and biofilm formation on the painted surface leading to cracking and disintegration of paint layers and degradation of binders resulting in detachment of the painted layer from the underlying support (Capodicasa et al., 2010; Ciferri, 1999; Felice et al., 2010; Pepe et al., 2010). Knowledge about microflora on the historical objects is of utmost importance to assessment and development of effective conservation strategies for management of microbial induced biodeterioration.

Conventionally, culture-dependent method was commonly utilized to isolate and identify the members in the microbial communities, but only less than 1% of the whole population can be detected by the techniques (Schloss and Handelsman, 2003). A vast majority of microorganisms (≥99%) in environmental samples cannot be cultured

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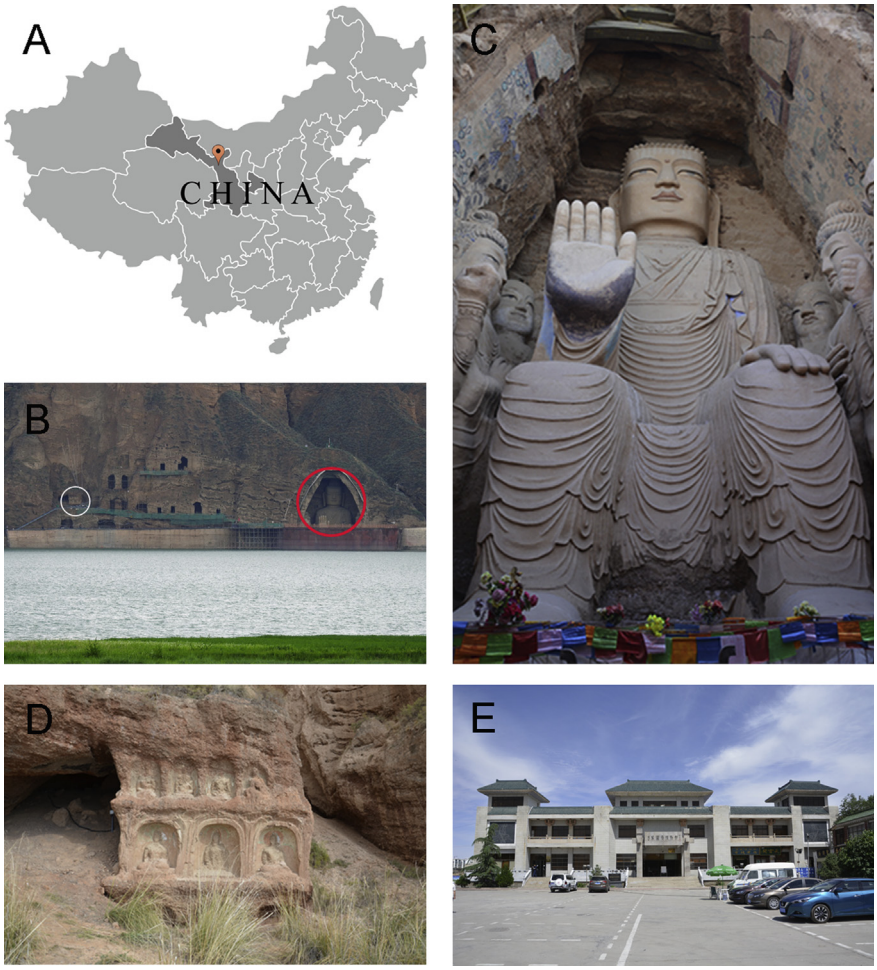

Fig. 1. Information of the research sites. A: location of the Tiantishan Grottoes in China; B: the survival caves in Tiantishan Grottoes, the Cave 13, namely, Grand Buddhist Temple (Red circle and C) and Cave 18 (White circle and D), they are all located on the upside of Huangyang Reservoir; E: the external landscape of the Wuwei Xixia museum. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

currently according to the procedures and the available methods only provide very limited information on the diversity and abundance of microbes thriving on heritage objects. To overcome the obstacles of the culture-based methods, modern molecular techniques are widely used for analyzing microflora on historical objects as a powerful tool nowadays, these approaches are mainly divided into three categories (Dakal and Arora, 2012): (i) clone library based techniques to obtain sequencing of 16/18S ribosomal DNA/RNA and ITS, (ii) community fingerprinting or profiling techniques such as DGGE, TGGE, t-RFPL, and (iii) next-generation sequencing and bioinformatics techniques. Recently, application of next generation sequencing method, such as Roche 454-pyrosequencing (Rosado et al., 2014) and Illumina MiSeq platform (Caporaso et al., 2012; Logares et al., 2014) provides additional insights into the microbial world on the historical objects (Duan et al., 2017; Li et al., 2016, 2017; Xu et al., 2017).

Previously, many reports were mainly concentrated on the microbes in several selective niches, such as the wall paintings preserved in caves, temples and catacombs (Capodicasa et al., 2010; Cuezva et al., 2012; Lan et al., 2010; Ma et al., 2015; Zucconi et al., 2012). These results revealed several core microbes associated with the deterioration of the cultural relics under similar conditions (Porca et al., 2012). Tiantishan Grottoes, a renowned Buddhist cave temple located on the ancient Silk Road in West China, was selected for this investigation. The Tiantishan Grottoes are known for their special historical status and magnificent Buddha statue. In 1950s, due to construction of the Huangyang River Reservoir and to avoid potential damages to the wall paintings or the painted sculptures at this site, conservation measures of removal of the wall paintings, and then transfer them to Gansu Provincial Museum for *ex situ* conservation and storage before final delivering them to Wuwei Xixia Museum in 2006 were taken. The rest of the painted sculptures are still left without intervention on their original location, namely, the outdoor environment. It should be noted that the *ex situ* conservation of wall paintings in particular is an active intervention measure used universally for protection of endangered cultural heritage in China. For example, the wall paintings of tombs are usually handled this way due to the poor burial conditions and unavailability of *in situ* conservation; infrastructure construction and economic development were often required for *ex situ* conservation of cultural relics. Undoubtedly, the *ex situ* conservation strategies provide a better condition for a long-term preservation of wall paintings. However, microbial growth and invasions can easily take place on the wall paintings during transportation process or long-term preservation under hostile or non-controlled environmental conditions. The physical state of wall paintings in the Tiantishan Grottoes has changed drastically after the long-term artificial environmental conditions were modified. In museum, severe microbial growth was detected with visible microbial filaments and spores on the surface of painted layer and even deeply penetrated into the back supporting layer. However, no apparent microbial damage was detected to those preserved *in situ* of the Tiantishan Grottoes.

To advance our current knowledge on the microorganisms responsible for the phenomenon observed; we adopted an innovative