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ORIGINAL PAPER

Diversity and seasonal dynamics of airborne bacteria in the Mogao Grottoes, Dunhuang, China

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Abstract The aim of this study was to analyze the phylogenetic composition of the bacterial community in the air at the Mogao Grottoes (Dunhuang, China) using a culture-dependent molecular approach. The 16S rRNA genes were amplified directly from the isolates with universally conserved and bacteria-specific rRNA gene primers. The PCR products were screened by restriction fragment length polymorphism, and representative rRNA gene sequences were determined and sequenced. A total of 19 bacteria genera were identified among 49 bacterial sequence types. Phylogenetic sequence analyses revealed high diversity within the bacterial community. The most predominant bacteria were *Janthinobacterium* (14.91%), *Pseudomonas* (13.40%), *Bacillus* (11.25%), *Sphingomonas* (11.21%), *Micrococcus* (10.31%), *Microbacterium* (6.92%), *Caulobacter* (6.31%), and *Roseomonas* (5.85%). The composition of bacterial communities differed greatly between different sites and at different times. The distribution of various bacteria was mainly affected by climatic parameters and human activities. These findings suggested that the opening of this cultural heritage site to visitors should be controlled and that maintaining the cave's natural climatic conditions would aid the conservation and management of the grottoes' paintings.

Keywords Airborne bacteria · Bacterial diversity · Bacterial community · Mogao Grottoes · Culturable bacteria · Cultural heritage

1 Introduction

Airborne microorganisms play a critical role in the biodeterioration of cultural heritage sites, as they are able to colonize almost any habitat on earth (Warscheid and Braams 2000; Woese 1994). Many environments contain diverse communities of microorganisms, and heritage sites such as caves containing historic artworks are not exempt (Gonzalez et al. 2008; Griffin et al. 1991; Schabereiter-Gurtner et al. 2004). In such sites, the growth of airborne microorganisms such as bacteria and fungi may affect the paint pigments and the underlying rock material, and many cases of biodeterioration of artworks have been reported all over the world, such as the famous Altamira cave, Lascaux cave, Tito Bustillo, and La Garma caves

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(Bastian et al. 2010; Dupont et al. 2007; Portillo et al. 2009; Schabereiter-Gurtner et al. 2002a, 2004). Information on microbial community structures in cave environments containing precious artworks is limited, but increasing our knowledge in this area is important with regard to understanding the impact of microorganisms on valuable heritage sites (Saiz-Jimenez and Gonzalez 2007).

Aerobiological investigations of cultural heritage sites may help to understand the process of biological degradation of artworks because the atmosphere is the main vehicle for the transportation and dispersion of microorganisms (Nugari et al. 1993). Constituents of the bioaerosol in the atmosphere may originate from both natural and anthropogenic sources, including vegetation and soil (Lighthart and Shaffer 1994), vegetables (Bovallius et al. 1978; Lindemann et al. 1982; Lindemann and Upper 1985), animal feeding (Wilson et al. 2002; Zucker et al. 2000), and traffic (Lugauskas et al. 2003; Sanchez-Moral et al. 1999; Wu et al. 2007). Understanding the microbial communities that reside in bioaerosols is important, and numerous studies have been conducted to determine the species composition and distribution of airborne microorganisms in many environments (Abdel Hameed et al. 2009; Fang et al. 2007; Lee et al. 2006; Lighthart and Shaffer 1995; Pepeljnjak and Segvic 2003; Tong and Lighthart 2000). Despite the use of a range of different study methods, the most commonly reported airborne bacteria genera are *Bacillus*, *Micrococcus*, *Staphylococcus,* and *Pseudomonas* (Fang et al. 2008; Shaffer and Lighthart 1997). However, most previous studies have focused primarily on organisms affecting human health (Tong 1999; Tong and Lighthart 2000), and few studies have evaluated the organisms present in the atmosphere of cave environments at cultural heritage sites. Microbiological studies in cave environments containing artworks are of interest because of the deterioration that microorganisms may cause (Dornieden et al. 2000). Recent publications have reported increased detection of microorganisms in cave environments, and microbial colonization has been observed in caves with cultural heritage, attracting the interest of scientists and conservationists (Gonzalez et al. 2003, 2006; Portillo and Gonzalez 2009; Saiz-Jimenez and Gonzalez 2007).

A year-round investigation was carried out previously, concerning the microbial concentration, the distribution of particle sizes, and community diversity in the air of four selected sampling sites in the Mogao Grottoes, Dunhuang, China (Wang et al. 2010a, b). The results regarding the microbial concentration and size distribution were reported (Wang et al. 2010a, b). In the present study, the major components of the microbial communities of airborne bacteria sampled from the Mogao Grottoes were explored, and our data provide a first insight into the microbial composition in such environments.

2 Materials and methods

2.1 Sampling site and sample collection

The Mogao Grottoes are located 25 km southeast of Dunhuang City, in the cliffs of the Daquan Valley. They span about 1,600 m north to south. The grottoes are considered an oasis in Gobi desert, located at a religious and cultural crossroads on the Silk Road, in Gansu Province, China.

Four sites were selected for the sampling of aerosols from September 2008 to August 2009, including three caves and one outdoor site. (1) The Open Cave (OC), numbered 16 by the local administration, has a volume of 1,744.7 m^3 and is situated in the northwest part of the Mogao Grottoes. It is the largest among the three sampling caves. This cave is open to visitors year-round because of the famous and much visited Library Cave, numbered 17, just inside. (2) The Semi-closed Cave (SC), numbered 244, has a volume of 243.3 m^3 and was the only sampling site located on the second tier of the cliff. This cave is only open to visitors in the peak tourist season and closed the rest of the year. (3) The Closed Cave (CC), numbered 54, is 101 m^3 in volume and is closed permanently for conservation purposes. During the sampling period, it was opened for sampling 1 day each month. (4) The Entrance (EN), about 5 m away from the nearest caves, is the area in which visitors' tickets are checked and many tourists wait for entrance to the caves. This is one of the most crowed places in the Mogao Grottoes.

Airborne bacteria were collected at each location using a six-stage culturable FA-1 sampler (similar to the Andersen sampler, made by the Applied Technical Institute of Liaoyang, China). Airborne particles were separated into six fractions, and the aerodynamic cut-off diameters in the six stages were

>7.0 μm (stage 1), 4.7–7.0 μm (stage 2), 3.3–4.7 μm (stage 3), 2.1–3.3 μm (stage 4), 1.1–2.1 μm (stage 5), and 0.65–1.1 μm (stage 6). Sampling was conducted from September 2008 to August 2009 at the four sampling sites in the Mogao Grottoes. At each of the sampling sites, the sampler was mounted 1.5 m above ground level. Air was drawn at 28.3 l/min to impact on the Petri dishes containing R_2A agar media. R_2A medium was prepared according to Reasoner and Geldreich (1985): yeast extract, 0.5 g; proteose peptone (Oxoid), 0.5 g; casamino acids (Difco), 0.5 g; glucose, 0.5 g; soluble starch, 0.5 g; K_2HPO_4, 0.3 g; $MgSO_4{\cdot}7H_2O$, 0.05 g; Sodium pyruvate, 0.3 g; agar, 15 g; and dematerialized water, 1,000 ml (pH7.2). Samples were collected for 5 min at a time, with three repetitions, and this was continued for two consecutive days each month. Exposed culture dishes were incubated for 48 h at 37°C.

2.2 Bacteria enumeration and identification

Colony forming units (CFU) on each plate were enumerated, and bacterial concentrations were expressed as CFU per cubic meter of air (CFU/m^3). The results were amended using formulas described previously (Fang et al. 2008). Approximately 100 bacterial colonies from the samples were identified using restriction fragment length polymorphism and 16S rRNA gene sequencing methods every month. A representative number of bacterial colonies (100) from each sampling time were selected randomly from the sampler plates to quantitatively estimate the kinds of bacteria found at each site, and culturable bacteria were analyzed.

DNA of the isolates was extracted using the chloroform–isoamyl alcohol extraction procedure (Zhou et al. 1996). The 16S rRNA genes of these isolates were amplified using the primer set: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′). The reaction mixture (25 μl) consisted of 1 unit of Taq polymerase (Tiangen Co., Beijing, China), 0.2 mM dNTPs, 2.5 μl of 10 × PCR buffer, 2.5 mM of $MgCl_2$, 0.2 μM of each primer, 2.5 μl (ca. 10 ng) of DNA template. The amplification program was as follows: initial denaturation at 94°C for 3 min, then 30 cycles of 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1.5 min, and a final extension step of 10 min at 72°C. PCR products were detected by electrophoresis in 1% agarose gels. The PCR products were digested by two enzymes, *Bsu*RI and *Hin*6I (MBI, Fermentas) and then differentiated into several clusters according to their spectral patterns. The isolates in each cluster were used for identification.

Cloning was performed with the pGEM-T Vector System (Tiangen Co., Beijing, China) following the manufacturer's protocol, and the ligation product was subsequently transformed into *Escherichia coli* DH5α cells, which allowed for blue–white screening. Transformants were plated on LB medium containing ampicillin (100 mg ml^{-1}), X-Gal (20 mg ml^{-1}), and IPTG (200 mg ml^{-1}). Positive clones were identified by PCR amplification with pGEM-T vector-specific primers (T7/Sp6) using the same program as that used for 16S rDNA amplification. Bacterial lysates of these expected clones were sequenced by the Shanghai Majorbio Bio-technology Company (Shanghai, China). The 30 sequences obtained (ca. 1,500 bp) were then analyzed using the National Center for Biotechnology Information (NCBI) Blast program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The most similar sequences were extracted from the GenBank database. A phylogenetic neighbor-joining tree, including the obtained isolates and their closest relatives, was constructed using the MEGA software 4.0. The sequences retrieved during this study can be accessed under the numbers HQ323413–HQ323461.

2.3 Data on environmental parameters

Meteorological data used in this study were provided by Dunhuang Academy. Their monitoring station is located on the top of the grottoes (N 40°02.261′, E 094°48.196′), and computerized hourly data from this station were available starting from August 1, 2008. Environmental parameters subjected to statistical analysis included temperature, relative humidity (RH), rainfall, solar radiation, wind speed, wind direction, and surface temperature. In addition, each cave has a monitor to provide temperature and RH data. The number of visitors was recorded by the ticket office. We used data on environmental parameters from an average of 10 days before and after the sample days, collected between 9:00 am and 17:00 pm daily.

2.4 Statistical analysis

All of the experimental data were analyzed using SPSS Version 16.0 (SPSS, Standard Version) for one-way analysis of variance. Non-metric multidimensional scaling (NMDS) analysis was calculated using PAlaeontological STatisitcs (PAST) version 2.03 (http://folk.uio.no/ohammer/past/). The relationships between the concentrations of airborne bacteria and environmental parameters were tested using the Pearson correlation.

3 Results

3.1 Diversity of the culturable bacteria community from four sites

A number of sequences were retrieved from culturable bacteria samples obtained from the Mogao Grottoes. A total of 49 sequences were classified into 19 different genera corresponding to the GenBank data. A phylogenetic tree of all sequences was constructed and their relative positions are shown in Fig. 1. *Proteobacteria* (54.24%) was the most frequently encountered bacterial phylum, followed by *Actinobacteria* (23.67%) and *Firmicutes* (22.09%) (Fig. 2). To aid the analysis, eight of the most frequently encountered bacterial genera were selected as the predominant bacteria phyla, with a coverage of over 80% of the total CFU. *Janthinobacterium* (14.91%) was the most predominant bacterial genus, followed by *Pseudomonas* (13.40%), *Bacillus* (11.25%), *Sphingomonas* (11.21%), *Micrococcus* (10.31%), *Microbacterium* (6.92%), *Caulobacter* (6.31%), and *Roseomonas* (5.85%). Other bacterial genera detected in the Mogao Grottoes were *Paenibacillus* (5.4%), *Kocuria* (4.84%), *Staphylococcus* (3.38%), *Planomicrobium* (2.01%), *Arthrobacter* (1.6%), *Luteimonas* (1.44%), *Naxibacter* (0.77%), *Ramlibacter* (0.34%), *Exiguobacterium* (0.04%), *Acinetobacter* (0.01%), and *Aerococcus* (0.01%).

The structure of culturable bacteria communities varied greatly between different sampling sites; the most prevalent bacterial genera are presented in Fig. 3. Significantly higher proportions of the genera *Janthinobacterium*, *Sphingomonas,* and *Microbacterium* were detected in the OC (18.16% of *Janthinobacterium*, 15.3% of *Sphingomonas*, 11.21% of *Microbacterium*) than those in the other three sites (13.08, 9.21, and 3.2% in the CC; 13.7, 8.47, and 9.91% in the SC; 13.16, 9.47, and 3.39% in the EN). The proportions of *Micrococcus* and *Caulobacter* in the CC (15.21%, 8.18%) and the EN (10.02%, 9.67%) were higher than those in the SC (5.65%, 3.71%) and the OC (6.69%, 4.04%). The proportions of *Pseudomonas* in the SC (18.6%) and the EN (17.35%) were higher than those in the CC (11.6%) and the OC (11.9%). The lowest concentration of *Roseomonas* was found in the EN (3.24%), compared with the CC (5.87%), the SC (7.0%), and the OC (6.29%). There was no significant difference in the proportion of *Bacillus* found among the four sites.

3.2 Monthly dynamics of the culturable bacterial communities

The proportions of the predominant culturable bacteria were analyzed monthly from September 2008 to August 2009 at the Mogao Grottoes in Dunhuang (Fig. 4). The concentration of *Janthinobacterium* peaked in October in the OC (110.33 CFU/m^3), but no obvious difference was found among the other months and sites. The same trend in distribution was found for the genus *Sphingomonas* (88 CFU/m^3). The genera *Pseudomonas* and *Microbacterium* showed similar monthly distribution curves, in which a double peak appeared in August (57.33 CFU/m^3, 47.67 CFU/m^3) and October (36 CFU/m^3, 71.33 CFU/m^3) in the OC. Higher concentrations of *Bacillus*, *Micrococcus,* and *Caulobacter* were observed in the OC (25.67, 42.67, 14.00 CFU/m^3) and the EN (25.33, 30.33, 24.33 CFU/m^3) in June. In the CC, *Bacillus* peaked in November (62.67 CFU/m^3) and July (38 CFU/m^3), *Caulobacter* peaked in August (36 CFU/m^3), and *Roseomonas* peaked in November (34.33 CFU/m^3). *Roseomonas* also showed had a higher concentration in October in the OC (40.67 CFU/m^3).

Using NMDS analysis, the similarity among sampling sites and months was calculated regarding genus-level distributions, and these data are shown in Fig. 5. Significant correlations were found between genera level community compositions and sites ($r = 0.470$, $p = 0.001$), as well as months ($r = 0.310, p = 0.032$). The diversity of bacterial communities was represented by the Shannon–Wiener diversity index and is shown in Fig. 6. The mean value of the diversity index differed little among the four sites (2.20 in CC, 2.16 in SC, 2.17 in OC, 2.07 in EN), but sharp fluctuations

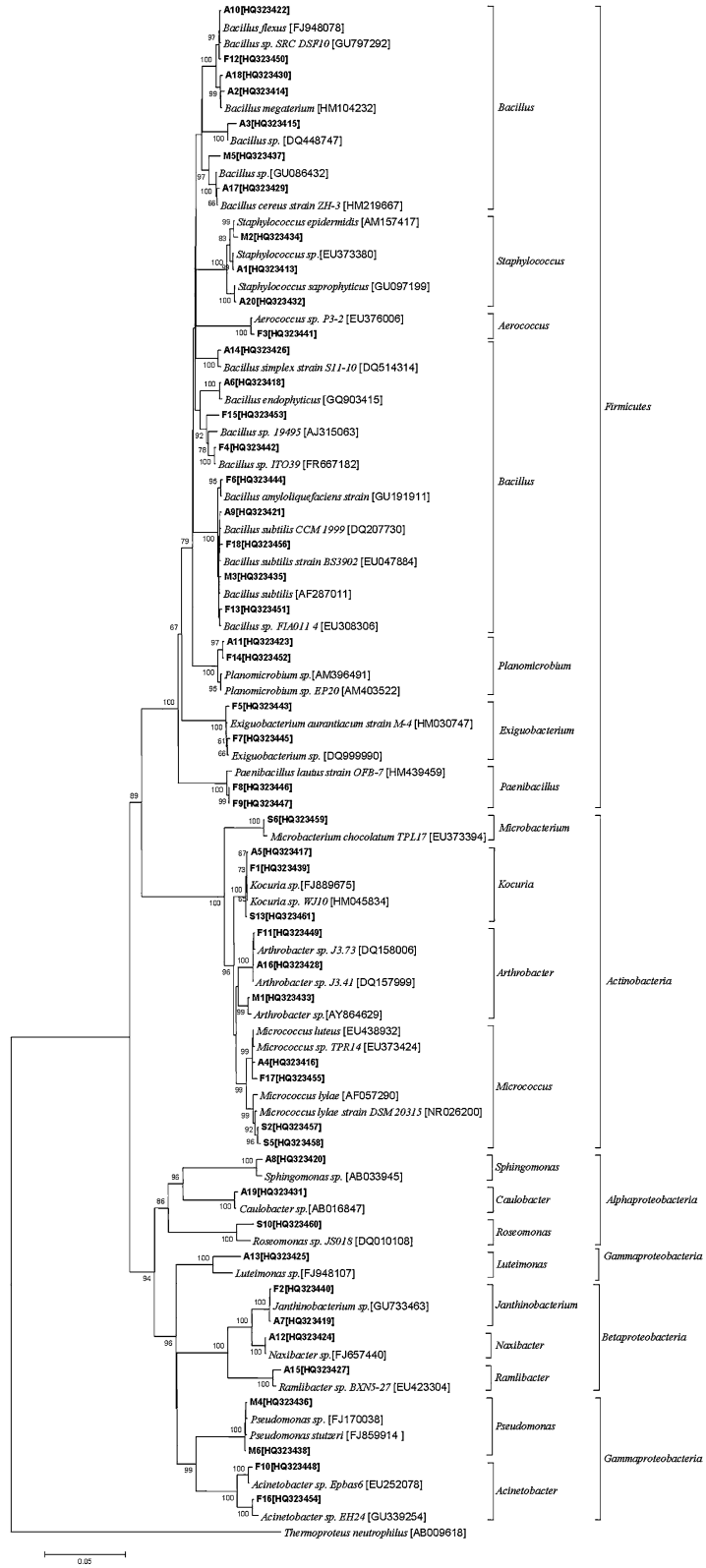

Fig. 1 Phylogenetic tree of bacterial 16S rDNA sequences derived from the airborne bacteria in the Mogao Grottoes. *Thermoproteus* was used as the outgroup. Bootstrap values represent 1,000 replicates, and only values greater than 60% are reported. The scale bar represents 0.05 substitutions per base position

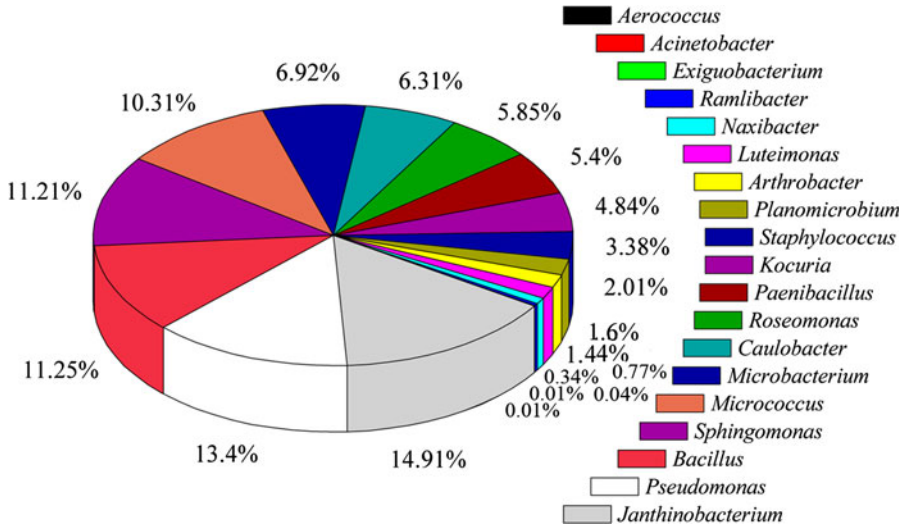

Fig. 2 Proportions of bacteria genera detected among the airborne bacterial community in the Mogao Grottoes

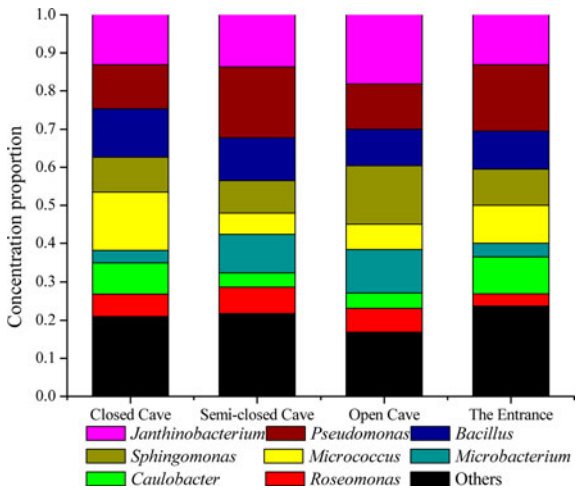

Fig. 3 Relative abundance of the predominant bacterial genera from the four sampling sites

were observed between months. A higher diversity index was detected in August in the CC (2.32), in June in the SC (2.33), in March in the OC (2.57), and in December in the EN (2.33). The lowest diversity index occurred in February in the EN (1.69).

3.3 Contributions of temperature, RH, and other environmental parameters to the structure of airborne bacterial communities

During the study period, most of the environmental parameters showed large differences among the four study sites (Table 1). Correlations between the airborne bacteria community composition and environmental parameters were determined by Pearson correlation analysis (Table 2). The number of visitors was positively correlated with the distribution of the most bacteria genera in the SC (*Microbacterium*), OC (*Sphingomonas*, *Microbacterium*, *Planomicrobium*, etc.), and the EN (*Planomicrobium*, *Pseudomonas*). *Caulobacter* and *Kocuria* were found to be more sensitive to temperature and solar radiation in the OC, SC, and EN. Rainfall and RH closely correlated with *Exiguobacterium*, *Micrococcus,* and *Staphylococcus* in the CC and SC. Wind direction had a significant influence on the distribution of *Naxibacter* and *Ramlibacter*.

3.4 Size distribution of airborne bacterial communities

The particle size distribution data of different airborne bacteria genera at the four sampling sites are shown in Fig. 7. Significantly higher bacterial proportions of *Janthinobacterium* and *Caulobacter* were observed for Stages 1, 2, and 3, contributing to 54.7 and 58.9% of their total concentrations, respectively. The genus *Roseomonas* was mainly distributed in the first four stages, with a proportion of 84.48%. *Micrococcus* was mainly distributed in Stages 5 and 6, contributing 52.07% to its total concentration. *Microbacterium* was mainly distributed in Stages 1, 3, and 4, *Pseudomonas* was mainly distributed in Stages 2 and 3, *Bacillus* was

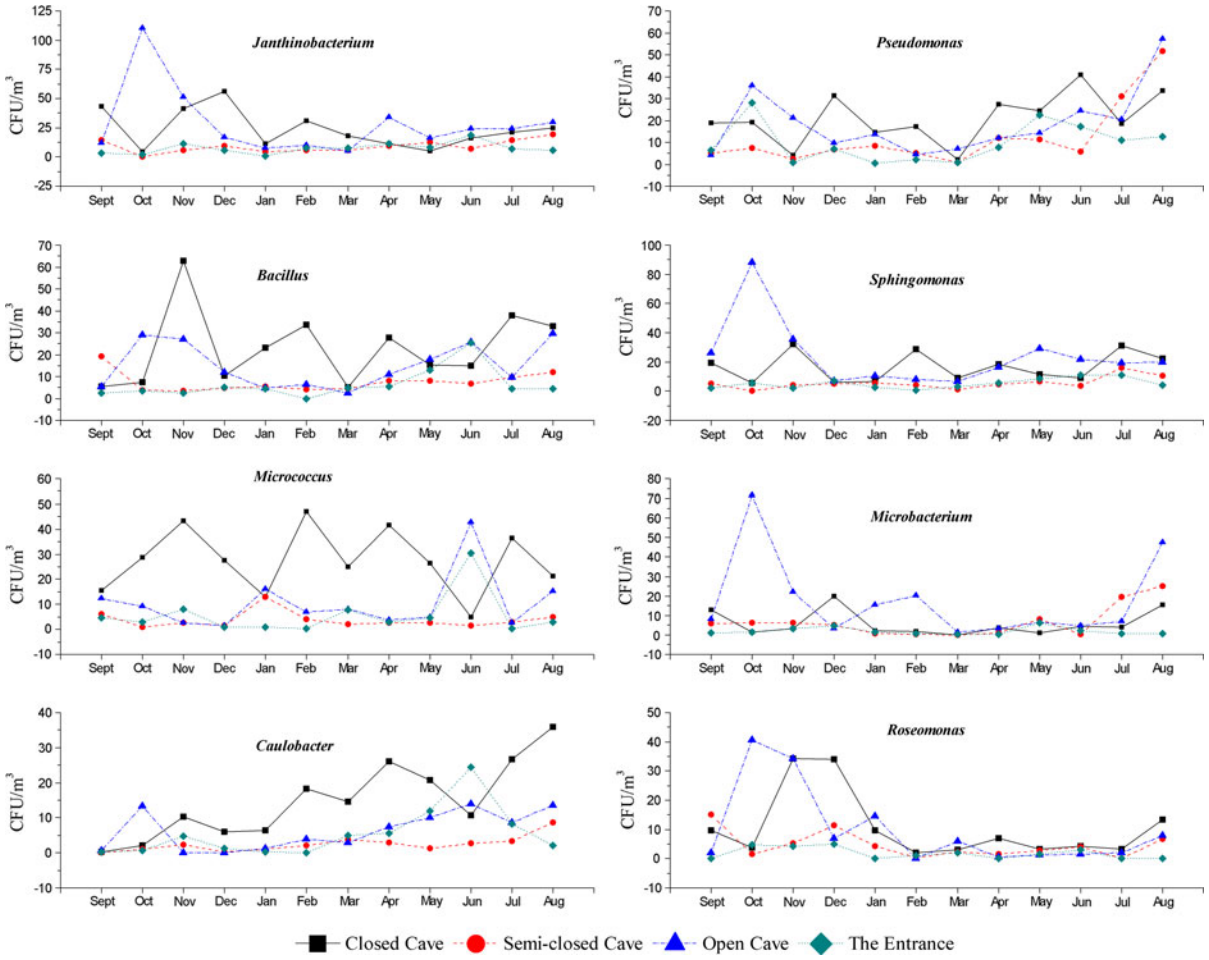

Fig. 4 Monthly variations in the predominant culturable airborne bacteria genera in outdoor air and in air from the *OC* open cave, *SC* semi-closed cave, and *CC* closed cave, expressed in CFU/m^3

mainly distributed in Stages 1 and 5, and *Sphingomonas* was mainly distributed in Stages 4 and 5. The size distribution patterns of the other less prevalent airborne bacteria also differed greatly among the six stages. *Staphylococcus*, *Ramlibacter,* and *Paenibacillus* were mainly distributed in Stage 6, *Arthrobacter* and *Kocuria* were mainly distributed in Stage 5, *Planomicrobium* was mainly distributed in Stages 3 and 4, *Luteimonas* was mainly distributed in Stage 4, and *Naxibacter* was mainly distributed in Stage 1.

4 Discussion

The desire to conserve unique cultural heritage sites has resulted in a number of studies focused on understanding the microorganisms thriving in caves holding important artworks. Both culture methods and DNA-based molecular approaches have been employed in recent years. In previous studies, *Actinobacteria* was the predominant phylum identified by culturing methods (Groth et al. 1999), while *Proteobacteria* was the bacteria phylum most frequently identified by 16S rDNA sequencing techniques (Schabereiter-Gurtner, Saiz-Jimenez, Pinar, Lubitz, and Rolleke 2002b). In this study, we detected about 54.24% *Proteobacteria* and 23.67% *Actinobacteria* as the major components of the bacteria community in the Mogao Grottoes. *Acidobacteria*, which was highly represented in a previous molecular survey (Schabereiter-Gurtner, Saiz-Jimenez, Pinar, Lubitz, and Rolleke 2002a), was not found

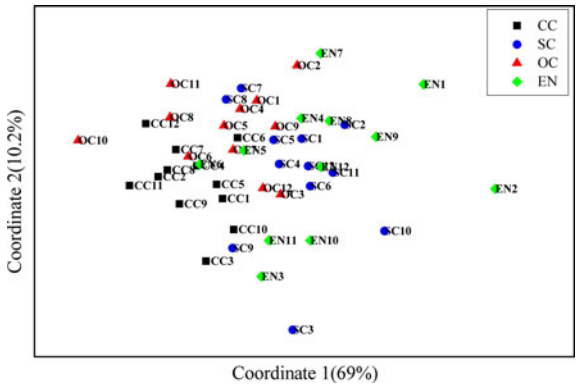

Fig. 5 Non-metric multidimensional scaling (NMDS) analysis based on Bray–Curtis similarity between the sampling sites at the *CC* closed cave, *SC* semi-closed cave, *OC* open cave, and the *EN* Entrance and the month (from January to December, numbered 1–12). Similarity was calculated using the genus-level community composition

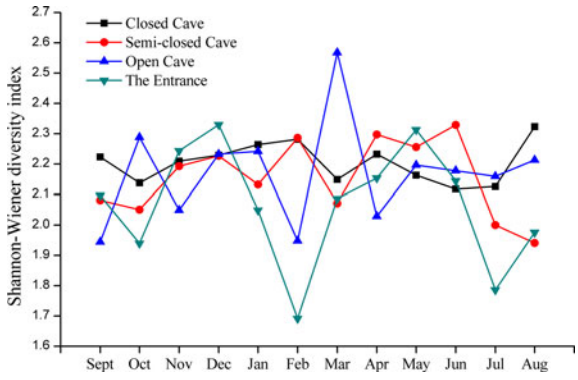

Fig. 6 Monthly distribution of the Shannon–Wiener diversity index for the different sampling sites

in this study. Laiz et al. (2003) reported that *Actinobacteria* and *Firmicutes* contributed to around 70% of the isolates obtained from Altamira cave, which also constituted 45.76% of the bacteria community in this study.

The airborne bacterial communities in the four sites analyzed in the present study showed differences in their structures, but no unique structure was found to be associated with a particular site. Although three genera (*Aerococcus*, *Acinetobacter*, and *Exiguobacterium*) were detected only in one or two sites (not in all four sites), their distributions among sites did not differ significantly. The diversity of the bacteria communities showed complex variations among the four sites (Fig. 6). However, a strong negative correlation was found between the diversity index in the CC and the outside temperature, as well as the wind speed. This result may indicate that the appearance of predominant bacteria would reduce the diversity of the bacteria community, and windy conditions would disturb the balance of the bacteria community in caves, potentially also reducing the diversity. High proportions of *Janthinobacterium*, *Sphingomonas,* and *Microbacterium* were detected in the airborne bacteria community in the OC, and their presence was closely correlated with the number of visitors (Table 1), suggesting that tourists are a possible source of airborne bacteria (Hoyos et al. 1998). The chemoorganotrophic bacteria *Caulobacter* constituted a large fraction of the bacterial community in the CC and the EN, showing seasonal distribution by its positive correlation with temperature and solar radiation. In oligotrophic environments

Table 1 Distribution of environmental factors in the Mogao Grottoes during 2008 and 2009

Environmental factors	Open cave (OC)			Semi-closed cave (SC)			Closed cave (CC)			The entrance (EN)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Temperature (°C)	17.28	6.42	26.74	12.62	2.93	21.81	10.77	2.39	18.26	13.55	−8.71	25.38
Relative humidity (%)	23.02	15.69	32.91	18.46	12.31	29.50	23.81	14.86	43.82	24.94	8.51	59.05
Solar radiation (kw/s*m^2)	–	–	–	–	–	–	–	–	–	0.50	0.23	0.84
Wind speed (m/s)	–	–	–	–	–	–	–	–	–	3.72	2.38	4.86
Wind direction	–	–	–	–	–	–	–	–	–	161.88	122.14	205.23
Rainfall (mm)	–	–	–	–	–	–	–	–	–	1.79	0	13.4
Surface temperature (°C)	–	–	–	–	–	–	–	–	–	22.77	−4.54	39.41
Visitors (people/month)	11,760	874	34,544	NA	NA	NA	–	–	–	11,760	874	34,544

Presented data were measured using the mean monthly values

NA data not available

Table 2 Pearson correlation analysis models for members of the airborne bacteria community and environmental parameters

Genera	Temperature	Relative humidity	Solar radiation	Wind direction	Rainfall	Surface temperature	Visitors
Sphingomonas	0.677*(OC)	NS	0.625*(EN)	NS	NS	NS	0.745**(OC)
Microbacterium	NS	0.667*(OC)	NS	NS	NS	NS	0.737**(OC) 0.657*(SC)
Planomicrobium	0.612*(CC) 0.720*(SC)	0.791**(SC)	NS	NS	NS	NS	0.626*(OC) 0.595*(EN)
Arthrobacter	NS	−0.716*(SC)	NS	NS	NS	−0.624*(CC)	NS
Caulobacter	0.622*(OC-EN)	NS	0.696*(OC) 0.647*(EN)	NS	NS	0.646*(OC)	0.720*(OC)
Kocuria	0.744**(SC-EN)	NS	NS	NS	NS	0.693*(SC)	0.577*(OC)
Exiguobacterium	−0.640*(CC-EN)	0.778**(CC-EN)	NS	NS	0.985**(CC)	NS	NS
Janthinobacterium	NS	–0.623*(EN)	NS	NS	NS	NS	0.644*(OC)
Micrococcus	NS	0.774**(SC-EN)	NS	NS	0.896**(SC)	NS	NS
Staphylococcus	NS	0.666*(SC-EN) −0.624*(EN)	NS	−0.708*(OC)	0.888**(SC)	NS	NS
Pseudomonas	NS	NS	0.647*(EN)	NS	NS	0.636*(EN)	0.741**(OC) 0.813**(EN)
Naxibacter	NS	NS	−0.701*(EN)	0.752*(SC) 0.646*(EN)	NS	NS	NS
Ramlibacter	NS	NS	NS	0.651*(OC)	NS	NS	NS
Luteimonas	NS	NS	NS	NS	0.689*(OC)	NS	NS
Paenibacillus	NS	NS	NS	NS	0.723*(CC)	NS	0.795**(OC)
Bacillus	NS	NS	NS	NS	NS	NS	0.650*(OC)

The capital letters in parentheses represent the sites in which the bacteria were distributed, *CC* closed caves, *SC* semi-closed cave, *OC* open cave, *EN* the entrance. *OC-EN* indicates that the bacterial distribution in the open cave was correlated with the environmental parameters in the entrance

NS no significant correlation

** Correlation is significant at the 0.01 level (two-tailed)

* Correlation is significant at the 0.05 level (two-tailed)

like caves, nutrients are a limiting factor for the growth of chemoorganotrophic bacteria (Barton and Jurado 2007; Laiz et al. 1999). These nutrients may be traced back to the seasonal activity of plants and insects (Bastian et al. 2009; Chelius et al. 2009; Jurado et al. 2008). The concentrations of *Micrococcus*, *Exiguobacterium,* and *Staphylococcus* increased during rainy conditions, the high air humidity leading to the growth of these genera. Low RH restricted the growth of these bacteria in the Mogao Grottoes, for its average RH was just 24% annually (Wang et al. 2010b). If the RH increased slightly in the Mogao Grottoes, these bacteria would cause serious bioerosive damage of the precious paintings. The wind direction was also found to influence the composition of the bacterial community in this study. The southeast wind reduced the concentration of *Naxibacter* and *Ramlibacter* in the SC and the EN, while the northwest wind increased their concentration. There were two possible interpretations of this phenomenon: the doors of the grottoes were southeast-facing, so airborne bacteria in the caves could not be diluted by the northwest wind; or the southeast wind often brought rainfall, which cleaned the air. However, the larger area of the OC may amortize the impact of the wind, and the location of this cave was lower than that of the SC. Many genera of bacteria detected in this study could produce yellow, red, and white pigments, including *Staphylococcus*, *Micrococcus*, *Planomicrobium,* and *Pseudomonas*. Furthermore, *Bacillus* and *Arthrobacter* were found to reduce hematite (iron oxide) in vitro (Gonzalez et al. 1999). It is therefore possible that all of these bacteria may have contributed to the color change observed in the Mogao mural.

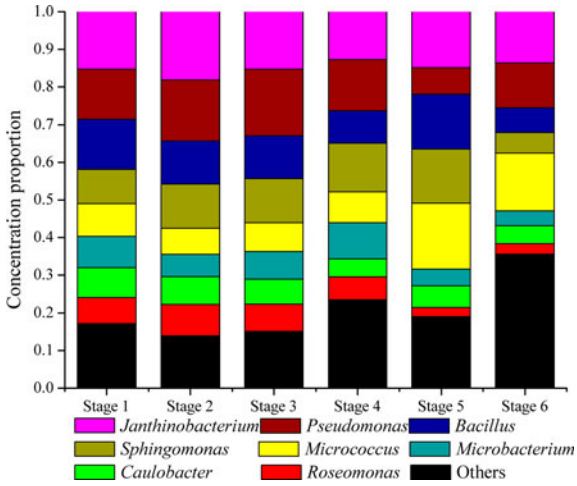

Fig. 7 Relative abundance of the predominant bacterial genera found for the six stages using the FA-1 sampler

The monthly distribution of predominant airborne bacteria varied greatly between different months. In general, the number of visitors had a significant impact on the distribution of *Janthinobacterium*, *Sphingomonas,* and *Microbacterium* in the OC, especially during the National Day vacations in October. Thus, the careful management of caves that are open to human visitation to control the number of visitors is of great importance, and maintenance of the caves' natural climatic conditions is also vital. The size distributions of bacteria during different stages also indicated the presence of different bacterial genera in the air. The bacteria *Janthinobacterium*, *Caulobacter*, *Roseomonas*, *Pseudomonas,* and *Bacillus* were all mainly distributed in the stages relating to trapping ranges larger than 3 μm. Bacteria are often attached to the surface of dust particles, and the size of individual bacterial particles is about 0.25 μm (Stanley and Linskens 1974). The actinomycetes, including *Micrococcus*, *Arthrobacter,* and *Kocuria*, were mainly distributed in the stages relating to trapping ranges smaller than 2 μm. In contrast to fungi, smaller spore particles (<5 μm) can penetrate the lower airways, leading to allergic reactions/asthma (Horner et al. 1995). Although this potential threat exists, the total concentration of organisms in Mogao Grottoes was low compared with most inhabited regions.

In summary, high bacteria diversity was found in the air of Mogao Grottoes in the present study. The structure of the bacterial community in different sites and during different months varied greatly. The distribution of various bacteria genera was limited by environmental parameters, as well as human activity. These results highlight the difficulty in understanding and interpreting the microbial structure of these communities, and consequently, more attention should be paid to the conservation of cultural heritage sites.

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