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Microbial Community Composition Analysis in Spring Aerosols at Urban and Remote Sites over the Tibetan Plateau

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Abstract: This study presents features of airborne culturable bacteria and fungi from three different sites (Lanzhou; LZ; 1520 m ASL, Lhasa; LS; 3640 m ASL and Qomolangma; ZF; 4276 m ASL) representing urban (LZ and LS) and remote sites (ZF) over the Tibetan Plateau (TP). Total suspended particle (TSP) samples were collected with an air sampler (Laoying 2030, China) on a quartz filter. Community structures of bacteria and fungi were studied and compared among three different locations. The average levels of bacterial load in the outdoor air ranged from approximately 8.03×10^1 to 3.25×10^2 CFU m^{-3} (Colony forming unit per m^3). However, the average levels of fungal loads ranged from approximately 3.88×10^0 to 1.55×10^1 CFU m^{-3}. Bacterial load was one magnitude higher at urban sites LZ (2.06×10^2–3.25×10^2 CFU m^{-3}) and LS (1.96×10^2–3.23×10^2 CFU m^{-3}) compared to remote sites ZF (8.03×10^1–9.54×10^1 CFU m^{-3}). Similarly, the maximum fungal load was observed in LZ (1.02×10^1–1.55×10^1 CFU m^{-3}) followed by LS (1.03×10^1–1.49×10^1 CFU m^{-3}) and ZF (3.88×10^0–6.26×10^0 CFU m^{-3}). However, the maximum microbial concentration was observed on the same day of the month, corresponding to a high dust storm in Lanzhou during the sampling period. The reported isolates were identified by phylogenetic analysis of 16S rRNA genes for bacteria and ITS sequences for fungi amplified from directly extracted DNA. Bacterial isolates were mostly associated with *Proteobacteria, Eurotiomycetes and Bacillus*, whereas fungal isolates were mostly *Aspergillus* and *Alternaria*. Overall, this is a pioneer study that provides information about the airborne microbial concentration and composition of three sites over the TP region depending on environmental parameters. This study provided preliminary insight to carry out more advanced and targeted analyses of bioaerosol in the sites presented in the study.

Keywords: bioaerosol; diversity; Tibetan Plateau; microbial community; culturable microorganisms

1. Introduction

Microorganisms in the aerosol are often considered as passive inhabitants of the atmosphere that are scattered via airborne dust particles, desert-sand and anthropogenic particles [1]. However, modern studies propose that many atmospheric microbes are metabolically active [2–4], even up to altitudes of 20,000 m [3,5]. Bioaerosol particles transport through the free troposphere to-and-from the Asian continental area to other up/downwind areas greatly influence climate change, ecosystem dynamics

and human health [1,6–9]. These atmospheric microbes need comprehensive investigation as it impacts human health in many ways, such as respiratory diseases [10–12]. The abundance and composition of airborne microbial groups vary across time and space [3,13–16]. Till to the date, the environmental conditions and factors influencing changes in microbial abundances are poorly characterized. However, several studies have reported data on microbial diversity in different conditions such as season, meteorological factors and altitude [6,17,18].

Atmospheric microbes are highly ubiquitous (density up to 10^3–10^6 cells per cubic meter of air), which are mainly emitted from soil, forest, terrestrial as well as marine environments, desert, agricultural/composting activities and anthropogenic activities [19,20]. These organisms can even survive in hostile conditions, such as desiccation, high altitude, scarcity of nutrients, ultraviolet (UV) radiation and temperature [21]. Interestingly, researchers have identified that the physical and chemical features of aerosols in the atmospheric boundary layer (ABL; from the surface to about 1–2 km high) are different from those in the free troposphere just above the ABL. Hence, it can be assumed that the microbial abundance and properties may also differ in different atmospheric layers. Bowers et al., (2012) have demonstrated the seasonal variability of airborne bacterial communities obtained in the remote site at Mt. Werner (3220 m ASL), USA [22]. Further, Tanaka et al., (2019) have reported the bacterial communities dominated by *Actinobacteria*, *Firmicutes* and *Proteobacteria*, while the eukaryotic communities Ascomycota, Basidiomycota and Streptophyta at a sub-urban site (23 m AMSL) in Toyama, Japan [20]. Although, past studies have not shown much significant difference in bacterial communities between the remote and sub-urban sites except for some less commonly found genus such as *Agaricomycetes* (Basidiomycota) at the remote site and *Dothideomycetes* (Ascomycota) at the suburban location. However, the findings from the study done by Tanaka et al., (2019) suggested that the bacterial and eukaryotic communities at the remote high-altitude site fluctuate more than at the sub-urban site [20] and local environmental factors influence the airborne eukaryotic community more than those on the airborne bacterial community, which are likely to be due to local vegetation type and weather condition [20,23,24].

Studies done worldwide have shown that bacterial concentrations differ among different types of outdoor milieus, with significant seasonal variations [25]. Similarly, many studies in the past have primarily recognized and presented the correlation between different weather conditions, meteorological factors and bioaerosol concentrations. The study conducted in Upper Silesia, Poland, presented the highest concentrations of airborne bacteria in outdoor air during spring [26]. Comparable result for higher bacterial concentration was observed in Colorado, USA and Montreal, Canada, during spring [16,22]. Bowers et al. (2012) accounted for the maximum average concentration of bacterial aerosol in the outdoor air during the spring season in Colorado [22]. Similarly, the annual bacterial distribution reported in the Montreal maxima in spring and autumn and minimal concentration in summer and winter [16]. This suggested the level of bacteria in the outdoor air vary with geographical region followed by seasonal change. However, the month or season cannot be regarded as the only criteria for seasonal patterns of airborne microbial variation, as many other factors influence the result. For example, the trend was opposite for the study done by Genitsaris et al. (2017) [27] who emphasized the higher concentration of bacterial aerosol in winter comparing to spring. Therefore, it is essential to explain the influence of meteorological parameters on the concentration and size distribution of bacterial bioaerosols in moderate climate zones. The present study was carried out in a typical Tibetan Plateau region, including remote and urban cities, to fill the knowledge gaps. It is challenging to collect aerosol samples in the remote Tibetan Plateau due to harsh environmental conditions and geographical patterns. Hence, the field campaign called "the second Tibetan Plateau Scientific Expedition and Research Program" was carried out during May 2019; therefore, the samples were collected during the spring period. In order to set a baseline and implement the research design, we examined the culturable bacteria, their concentration levels and community composition during a month of the spring season. This work was concentrated on culturable bacteria only because these microorganisms are very sensitive and seem to be highly influenced by a variety of meteorological factors [26]. Several

studies have successfully used a culture-dependent method for analysis of bioaerosol and established a general baseline dataset about culturable microorganisms in several sites [28,29]. A culture-dependent method is a standardized method (e.g., ISO 337 methods) that are usually considered the reference analytical methods for official controls [26,28–31]. Thus, to enhance the understanding of atmospheric dynamics under different geographic and environmental conditions, a precise investigation relating to environmental factors and bioaerosols is needed.

In this study, the population of airborne bacterial and fungal communities in total suspended particle (TSP) samples were simultaneously collected and evaluated from urban and remote sites of the Tibetan Plateau (TP) region. 16S and ITS gene hypervariable were evaluated to identify the bacterial and fungal isolates, respectively. We believe that this is the first-ever study done to investigate the type and abundance of bacterial and fungal communities in aerosol samples simultaneously collected at urban and remote sites over the TP zone. Moreover, this study can increase awareness of the influence of bioaerosols on human health, as well as provide references for a better understanding of OAQ (outdoor air quality) in the remote and urban areas of the TP region.

2. Materials and Methods

2.1. Sampling Site Description

TSP samples were collected at three sites: Qomolangma (Mt. Everest) (ZF; 28.36° N, 86.95° E, 4276 m ASL) Atmospheric and Environmental Observation and Research Station, Lhasa station (LS; 29°38′ N, 91°38′ E, 3640 m ASL.) in the campus of the Institute of Tibetan Plateau Research (Lhasa branch) and Lanzhou station (LZ; 36°3′1″ N, 103°51′33″ E, 1520 m ASL) on the roof of the building No. 3 at the Northwest Institute of Eco-Environmental and Resources, Chinese Academy of Sciences (Figure 1), based on the Atmospheric Pollution and Cryospheric Change (APCC) observation network [32]. The sampling sites were denoted as LZ: Lanzhou, LS: Lhasa, ZF: Qomolangma station. The Qomolangma (Mt. Everest) region is a typical representative of the remote site over TP in terms of climate, air circulation systems and environmental characteristics [33]. This site is relatively isolated from industrial zones and cities, with a minimal local population due to its harsh environment [34]. The city of Lhasa is located in a narrow west-east valley in the southern part of the TP. The sampling site is close to one of the busiest roads in the city, Jinzhu Road. There is a considerable coal-fired power and cement factory approximately 10 km to the west of the sampling site [35]. Moreover, Lhasa is a famous tourist–historic city and leads to significant seasonal variations in traffic and religious activities [36,37]. The climate of Lhasa is characterized by a wet summer monsoon season and a dry non-monsoon season. During the monsoon season (July through September), low pressure over the TP attracts warm air masses from the Indian Ocean into the plateau. While in other seasons (non-monsoon), the large-scale atmospheric circulation patterns over the TP are mainly dominated by westerlies [38]. The Lanzhou station located at the center of the valley and can be used to reflect the atmospheric aerosol conditions in urban areas of Lanzhou. Lanzhou has a typical semi-arid continental climate, with scarce precipitation (annual average of 250–350 mm), abundant sunshine and a significant temperature difference between day and night [39]. Its annual average temperature is 10 °C. There are usually intense sandstorms in winter and spring [40], influenced by the arid areas around Lanzhou (especially along the Hexi Corridor). Lanzhou is a typical dry valley city where the diffusion and transportation process of air pollutants is more complicated than in plain cities [41].

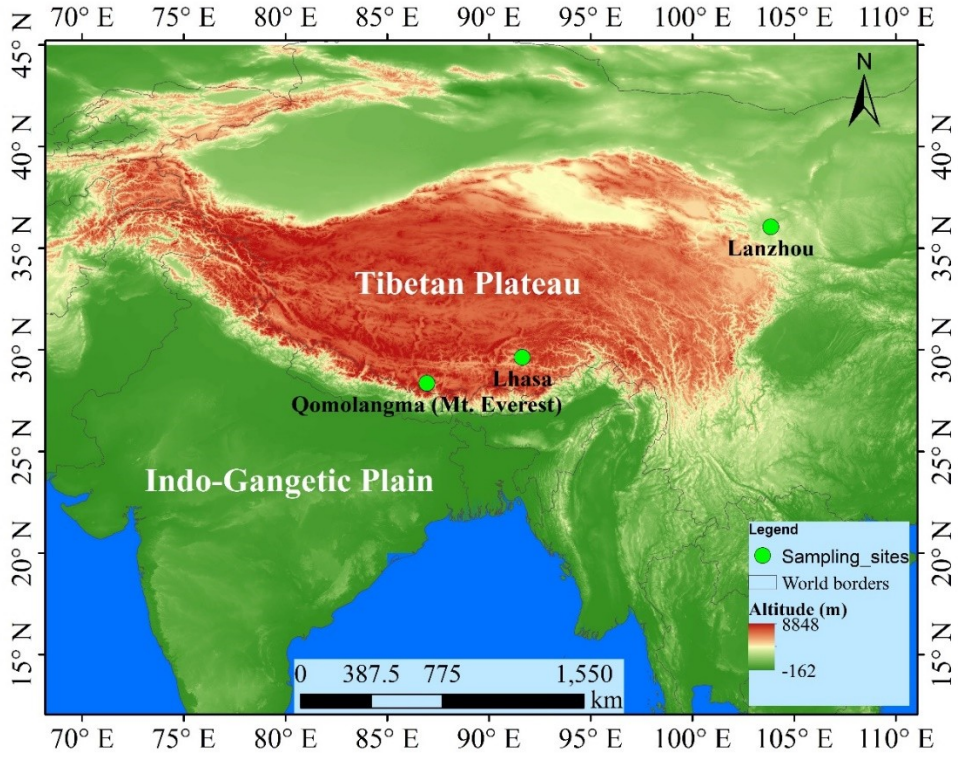

Figure 1. Geographical map for three sites presented in the study.

2.2. Samples Collection

An aerosol sampler (Laoying 2030) was used to collect TSP samples onto a quartz fiber filter 90 mm in diameter with 0.22 μm pore size (Whatman™, GE Healthcare, Chicago, IL, USA). TSP refers to the entire aerosol size range (broad range of particle sizes including fine, coarse and super coarse particles) ranging in size from 0.1 μm to about 30 μm in diameter. The flow rate was adjusted to 100 L/min, for 23 h (9:00 a.m. to 8.00 a.m. on the next day). Except the samples were collected for 48 h in Qomolangma station. Before use, the filters were sterilized in a muffle furnace at 550 °C for 5 h. The sample holder was sterilized by 75% ethanol between two samplings. Eight samples were collected from 1 May–22 May 2019, in each of the stations. A control sample was collected by putting a blank filter in the sampler, with the pump shut down for 5 min. All of the samples were stored at −20 °C until subsequent analyses.

2.3. Microbiological Analysis

For microbiological analysis, total viable cell count and culture identification were performed. The quartz filter membrane sample 27 mm was suspended in a 10 mL of sterilized normal saline (0.9% *w/v* of NaCl), and the suspension was diluted up to 10^{-7} times. For the enumeration of bacterial and fungal loads, 100 μL from each suspension was inoculated on nutrient agar medium for bacteria and potato dextrose agar (PDA) plates for fungi [42–45]. The nutrient agar plates were incubated at 37 °C for 48 h and PDA plates were incubated at 25 °C for 72 h [46]. Total viable cells were estimated as colony forming units (CFU) per mL/m^3. Bacterial and fungal colonies with different morphology and pigments were selected and purified several times on respective media to obtain pure cultures. The isolates were identified and characterized based on morphologic features and ribosomal RNA gene sequence analysis (16S rRNA for bacteria and ITS1 for fungal isolates).

2.4. DNA Extraction and Phylogenetic Analysis

The purified bacterial and fungal colonies that appeared on plates were first analyzed for their morphologic characteristics and subsequently harvested for genomic DNA extraction. Genomic

DNA extraction kits were used according to manufacturer instructions for bacterial DNA extraction (Tiangen Biochemical Technology (Beijing) Co., Ltd.) and fungal DNA extraction (Flying Biological Engineering (Guangzhou) Co., Ltd). The obtained genomic DNA was resuspended in 70 µL TE buffer blended with RNase and its quality was checked on agarose gel 0.8% (*w/v*) and stored at 4 ± 0.5 °C for subsequent analysis.

Molecular identification of bacterial and fungal isolates was carried out by sequencing the 16S rRNA gene of the bacterial isolates and ITS gene of fungal isolates. The genomic DNA from bacterial isolates was used for the full-length amplification of the 16S rRNA gene by using primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [47]. In the case of fungal DNA, the internal transcribed spacer 1 (ITS1) of the fungal rRNA gene was amplified by using primers ITS1F (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) [48].

A PCR reaction mixture of 50 µL was used consisting of 2 µL DNA, 2 µL deoxynucleotide triphosphate (dNTP), PCR buffer 5 µL, 1 µL each reverse and forward primer, 1 µL Ex Taq DNA polymerase, and 38 µL ddH_2O. The PCR reaction mixture was incubated at 94 °C for 5 min, and 35 cycles of amplification were completed at 94 °C for 30 s, 58 °C for 45 s, 72 °C for 80 s. Additionally, the reaction was incubated at 72 °C for 7 min. The control reaction was run without genomic DNA to confirm the accuracy of PCR amplification and sample purity. Each DNA sample was tested three times, and the obtained PCR products were combined. The PCR products were separated by using 1% agarose gel electrophoresis and purified with Axygen nucleic acid purification kit (Axygen, Biosciences, CA, USA) [49]. The purified PCR products (using AxyPrep DNA Gel Recovery Kit) was used for sequencing using a sequencer ABI3730-XL. The sequencing was performed on MATABIO company PR China.

The obtained sequences were checked for the homology sequences through the BLAST (basic local alignment search tool) search method in the National Center for Biotechnological Information (NCBI), and the strains were identified with 97% cutoff value at the species level. For phylogenetic analysis and tree construction, related sequences of all the species were obtained from NCBI and aligned by MUSCLE through MEGA6. Further, the obtained nucleotide sequences were deposited in the NCBI database and assigned with the accession number as MN840035-MN840042 for bacterial 16srRNA sequence and MN911298–MN911313 for fungal ITS sequence.

2.5. TSP Mass and Back Trajectory Analysis

The collected TSP filter weight was measured twice before and after the sampling and the net accumulation mass for each filter was calculated as the difference between the pre and post sampling weight microbalance after equilibration at constant temperature and humidity (20 °C, 39%) for 24 h. Field blank filters will also be collected through exposure to the sampler with no air drawn [50]. A five days air mass backward trajectory analysis was conducted to determine the air mass history at sampling sites during the sampling period, using the NOAA HYSPLIT4 model [51].

3. Results

3.1. Meteorological Conditions during the Sampling Period

The meteorological parameters, including temperature and relative humidity (RH), wind direction and wind speed (WS) were obtained from http://data.cma.cn/site/index.html. The meteorological information at three different sites during the sampling period is given in Table 1. As shown in Table 1, the temperature and pressure at Lanzhou and Lhasa stations were higher than at Qomolangma station. However, RH and WS were comparably higher in Lhasa and Qomolangma station than Lanzhou station, except for some specific days, where the RH and WS fluctuate in Lhasa and Qomolangma station. It shows that the RH and WS at the remote Qomolangma site were higher than that at the urban site (Lanzhou and Lhasa) and vice versa for temperature and pressure. The weather data obtained from http://tianqihoubao.com/lishi/lanzhou/month/201904.html showed 4 days of cloud and dust dated

12th, 13th, 15th and 18th of May 2019. Wind speed and direction are important factors that influence the movement of atmospheric aerosol particles and their mass concentration. The wind rose plots (Figure 2) indicate that the higher frequency of wind arrived mainly from W and WSW at the remote site (Qomolangma), while the tendency was different for the urban site (Lanzhou and Lhasa). At the site, westerlies predominantly prevailed owing to higher wind speed frequency up to 40%. Compared to other sites, it was also observed that high wind speeds reached 15.6 m/s, but less in frequency. The maximum wind speed at Lasha measured to be 9.6 m/s. The higher wind speed was typically from the west-north-west direction at the station site, whereas less speed observed up to 25% by frequency. At Lanzhou, the predominant wind blowing from NE followed by south direction with maximum wind speed 5.4 m/s. No precipitation was observed at each station during the sampling period.

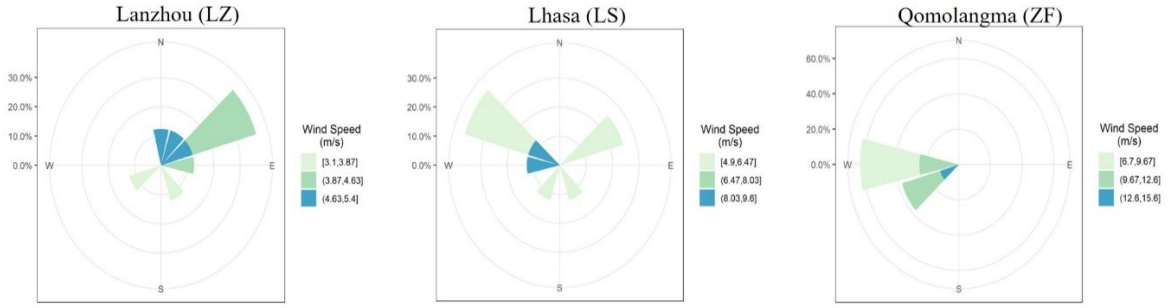

Figure 2. Windrose plot for three sites presented in the study. Windrose plot showing the frequency of wind speed and wind direction at three sites during the study period.

The TSP mass concentrations across the three study sites are presented in Table 1. The TSP mass in Lanzhou, Lhasa and Qomolangma ranged from 46–1530 μg m^{-3}, 68–199 μg m^{-3} and 32–130 μg m^{-3}, respectively. The statistical correlation analysis (Supplementary Tables: Tables S1–S3) revealed that the bacterial and fungal loads correlated well with WS in Lanzhou, suggesting wind speed could have influenced bioaerosol load in Lanzhou city. In contrast, in Lhasa and Qomolangma, no such significant correlations were observed between Bacterial and fungal loads with meteorological parameters inferring the influence from other factors such as agricultural or industrial impact and human activity. Both bacterial and fungal load did not show any relation with TSP mass at all sites attributing that other aerosols mostly influenced TSP mass (e.g., dust, biomass, anthropogenic factors) rather than bioaerosols. The five days air mass back-trajectories were executed for all sites during the sampling days and presented in Figure 3. The air mass coming from South Asia (especially Nepal and India) may have influenced the aerosol mass and bioaerosols as from the back-trajectories analysis at Qomolangma and Lhasa sites. Meanwhile, the air mass in Lanzhou was originated from western regions. However, long-term spatial studies are needed in the future to better understand the bioaerosol properties, sources and transport over the region.

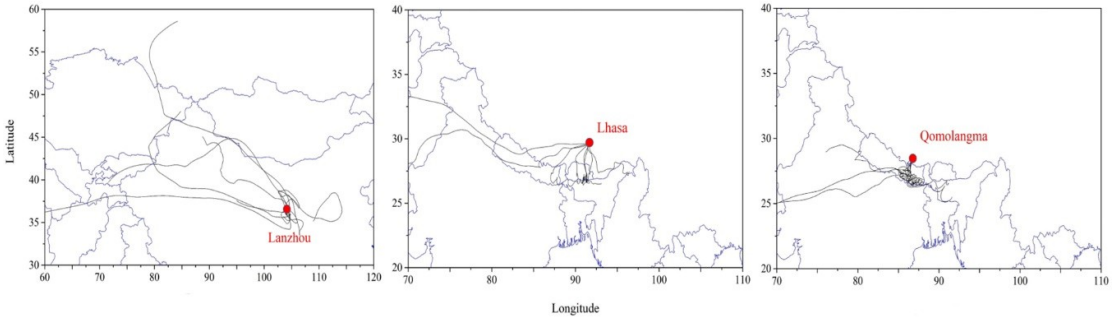

Figure 3. HYSPLIT backward-trajectory analysis for three sites Lanzhou (LZ), Lhasa (LS), and Qomolangma (ZF) during the sampling period.

Table 1. Summary of meteorological information of three sites Lanzhou (LZ), Lhasa (LS) and Qomolangma (ZF) and chemical composition of bioaerosol during the sampling period (May 2019).

	Temperature (°C)			Pressure (hPa)			RHU (%)			Wind Speed (m/s)			Wind Direction			TSP (μg m^{-3})		
Date	**LZ**	**LS**	**ZF**	**LZ**	**LS**	**ZF**	**LZ**	**LS**	**ZF**	**LZ**	**LS**	**ZF**	**LZ**	**LS**	**ZF**	**LZ**	**LS**	**ZF**
5/1/2019	21.1	22.7	15.8	831.1	661.9	605.5	32.9	89.9	49.9	3.5	5.6	10.9	SW	WNW	WSW	58.01	129.26	90.35
5/4/2019	25.1	19.4	18.6	832.3	660.9	603.8	22.9	32.9	18.9	4.4	4.9	6.7	E	ENE	W	72.05	96.49	40.39
5/7/2019	21	25.5	15.6	834.8	658	603.5	31.9	99.9	99.9	4.7	5.9	10.3	NE	SSW	W	1530.54	170.77	61.49
5/10/2019	18.1	24.2	18.4	833.4	658.2	605	36.9	59.9	69.9	4.5	9.2	11.8	NE	W	W	1521.63	153.86	72.15
5/13/2019	23.3	19	12.7	834.5	658.9	604	19.9	18.9	15.9	4.5	9.6	8.9	ENE	WNW	W	673.59	199.31	30.36
5/16/2019	25.7	20	16.7	830.7	660.9	604.9	23.9	21.9	12.9	5.4	5.1	10.3	NNE	SE	WSW	60.99	68.72	32.43
5/19/2019	18.7	22.2	16.2	839.7	659.3	604.8	21.9	16.9	99.9	5.4	5.3	8.5	N	WNW	W	225.84	104.36	33.93
5/22/2019	28.1	18.6	16.8	833.8	663.1	605.7	69.9	25.9	89.9	3.1	5.1	15.6	SE	NE	WSW	40.84	116.68	130.05

3.2. Airborne Microbial Community Abundance

Figure 4a represents the average load of bacteria in the outdoor air in Lanzhou, Lhasa and Qomolangma region during the study period. The first observation to be made is that the average load of the total bacteria collected over the three sites are significantly correlated. The average levels of bacterial loads in the outdoor air ranged from approximately 8.03×10^1 to 3.25×10^2 CFU m^{-3}. However, the average levels of fungal loads ranged from approximately 3.88×10^0 to 1.55×10^1 CFU m^{-3}. The maximum bacterial loads were observed for Lanzhou (3.25×10^2 CFU m^{-3}), followed by Lhasa (3.23×10^2 CFU m^{-3}) and Qomolangma (9.54×10^1 CFU m^{-3}). Similarly, the maximum fungal loads were observed for Lanzhou (1.55×10^1 CFU m^{-3}) followed by Lhasa (1.49×10^1 CFU m^{-3}) and Qomolangma (6.26×10^0 CFU m^{-3}). During the sampling period, dust-storm event was recorded in meteorological data (http://tianqihoubao.com/lishi/lanzhou/month/201905.html) in Lanzhou dated on 12th, 13th, 15th and 18th of May 2019. Similarly, the microbial loads were observed to be relatively higher on almost the closest sampling day of the month (16th, 19th and 22nd of May 2019). This observation led to consider a possible linkage between the dust storm and microbial load in Lanzhou, which could have somehow influenced the microbial concentration. Although, higher microbial load was also observed on the 7th and 10th day of the sampling period, the difference in microbial population makeup at three different sites (Figure 4b) suggests that several factors such as geographic location and weather could also influence the microbial loading and composition. However, further studies with more sample size are necessary to understand the interrelation between dust storm events with microbial load, their survival and transport. The control samples collected during the sampling period were also analyzed for the total viable count and microbial load. The culture plate did not produce any visible colonies and also could not be used for DNA extraction. This could not provide any comparative analysis with microbial loads obtained from samples. In some way this suggests that sterilization and sample handling process was void of contamination, while also to be noted the possibility of low microbial trapping amid control sampling period, however, this justifies further investigation of the situation and the requirement to take action [26].

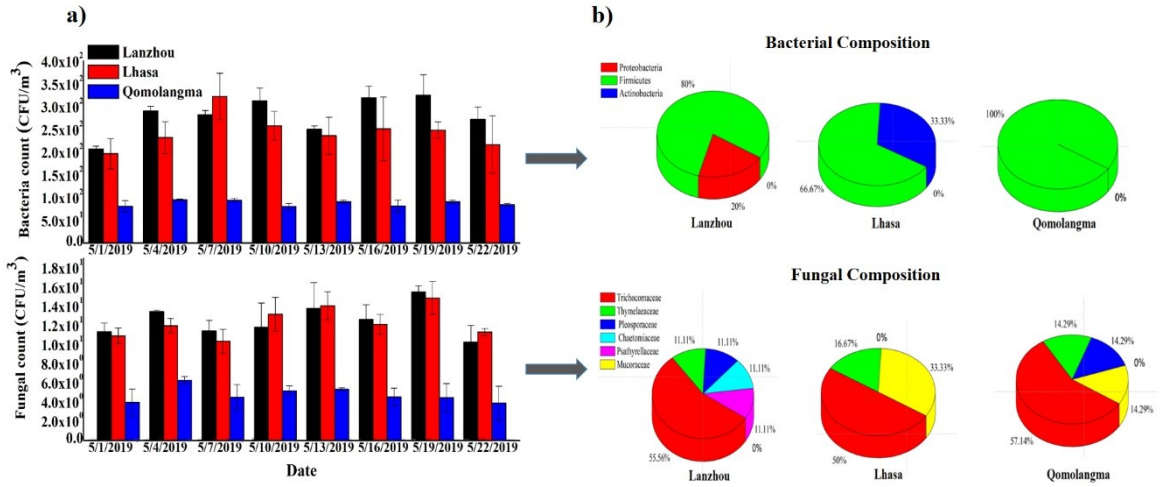

Figure 4. Concentrations and composition of bioaerosol present in the outdoor air in Lanzhou, Lhasa and Qomolangma site during May 2019. (**a**) average concentrations of bacterial and fungal aerosol collected in the outdoor air in Lanzhou, Lhasa and Qomolangma region during the analyzed period; (**b**) The composition of bacterial and fungal communities in the aerosol samples from Lanzhou, Lhasa and Qomolangma region during the study period.

3.3. Airborne Microbial Community Composition

The three sites are quite different in terms of aerosol loadings, which could have some impact on microbial loadings as well. This was observed in this study when the microbial composition was analyzed for three sites, as shown in Figure 4b. The bacterial community was dominated by members within *Firmicutes* in all three sites (LZ: 80%, LS: 67.67% and ZF: 100%) followed by *Proteobacteria* (20%) in LZ and *Actinobacteria* (33.33%) in LS. Similarly, the fungal community included members belonging to *Trichocomaceae* (more than 50% in all the three sites), followed by *Mucoraceae* (33.33% in LS and 14.29% in ZF) and *Thymelaeaceae* (more than 10% in all three sites), whereas *Pleosporaceae, Chaetomiaceae* and *Psathyrellaceae* were dominated only in LZ and ZF (more than 10%) (Figure 4b). This observation suggests the possible variation of microbial composition based on geographic location, followed by environment parameters that indirectly impact on transport and survival of microorganisms in ambient air.

Based on the sampling and sequencing method, the culturable microorganisms obtained showed diversity and richness congruent with previous culture-independent studies in airborne bacterial communities from rural and semi-urban regions [52–55]. In a comparison of the microbial community identified at different regions worldwide, including longitude, latitude, elevation and method used for the study are elaborated in Table 2. It can be observed that *Proteobacteria* and *Bacillus* are predominant above 1100 m ASL and *Bacillus*, *Eurotiomycetes* and *Zygomycetes* are predominant above 3200 m ASL However, following previous findings, it cannot be generalized that the community mentioned above is specifically dominant in given regions because the microbial community composition in the aerosol is found to be unstable in regards to geographical location.

Table 2. Comparison of the microbial community of viable bioaerosols in different regions worldwide.

Location	Latitude (°N) and Longitude (°E)	Elevation (AMSL)	Site	Method Used for Study	Microbial Community	References
Toyama Prefecture, Japan	36°41′54′′ N, 137°11′13′′ E	23 m	Suburban	Polycarbonate filters, Illumina sequencing	*Alpha-Beta-Gammaproteobacteria, Acidimicrobia, Planctomycetia, Bacillus, Solibacteres, Flavobacteria*	[20]
Huairou, Beijing, China	40°24′29″ N, 116°40′ 28″ E	40–60 m	Peri-urban	Quartz filters, Illumina sequencing	Streotophyta, *Bacillus, Clostridium, Kocuria, Staphylococcus, MethylobacteriumSarcinomyces, Trichothecium, Acromonium, Chaetomium, Aspergillus, Penicillium*	[18]
New Delhi city, India	28°12′ N–28°53′ N, 77°50′ E–77°23′ E	218 m	Urban	Quartz filters, automated DNA sequencing	*Bacillus, AcenitobactorAspergillus, Cladosporium, Alternaria, Fusarium, Penicillium, Trichoderma, Mucor*	[17]
Jawali, India	31°2′ N–32°5′ N 75°0′ E–77°45′ E	600 m	Rural	Quartz filters, Light microscopy	*Basidiospora, Ascospora, Fusarium, Ganoderma, Alternaria, Curvularia*	[12]
Erenhot	43.668, 111.953	957 m	Urban	Polycarbonate filters, Illumina sequencing	*Chloroacidobacter, Saprospirae, Actinobacteria, Alphaproteobacteria, BacilliAgaricomycetes, Dothideomycetes, Sordariomycetes, Eurotiomycetes*	[9]
Qingdao, China	36°16′ N, 120° 50′ E	1133 m	Urban	Six stage cascade impactor, DGGE band sequencing	*Alphaproteobacteria, Betaproteobacteria, Bacillus*	[6]
Lanzhou	36°3′1″ N, 103°51′33″ E	1520 m	Urban	Quartz filters, Illumina sequencing	*Proteobacteria, BacillusEurotiomycetes, Malvales, Dothideomycete, Sordariales, Agaricales*	This study

Table 2. *Cont.*

Location	Latitude (°N) and Longitude (°E)	Elevation (AMSL)	Site	Method Used for Study	Microbial Community	References
Salento's peninsula, Italy	40.3° N; 18.1° E	1895 m	Suburban	PTFE filters, Illumina sequencing	*Proteobacteria, Cyanobacteria, Actinobacteria, Pseudomonas, Enterobacter, Vibrio, Streptomyces*	[7]
Mt. Jodo, Japan	36°34′00′′ N, 137°36′21′′ E	2839 m	High altitude, pristine	Polycarbonate filters, Illumina sequencing	*Alpha/Beta, Gammaproteobacteria, Acidimicrobia, Planctomycetia, Bacillus, Solibacteres, Flavobacteria*	[20]
Sierra Nevada, Spain	37°03′ N, 3°23′ W	2896 m	High altitude, Rocky and meadows	Passive automatic sampler	*Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria*	[8]
Noto Peninsula, Japan	Uchinada (36°67 N, 136°64 E) to Hakui (36°92 N, 136°76 E)	500–3000 m	Coastal	Polycarbonate filters, Illumina sequencing	*Cyanobacteria, Actinobacteria, Bacillus, Alpha, Beta, Gammaproteobacteria*	[1]
Mt. Werner, Colorado, USA	40.45° N, 106.73° W	3200 m	High altitude, pristine	Cellulose nitrate filters, Sanger sequencing	*Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Bacillus, Eurotiomycetes, Sordariomycetes, Dothideomycete*	[3]
Lhasa	29°38′ N, 91°38′ E	3640 m	High altitude, Peri-urban	Quartz filters, Illumina sequencing	*Bacillus, Kocuria, Zygomycetes, Eurotiomycetes, Malvales*	This study
Qomolangma	28.36°N, 86.95°E	4276 m	High altitude, Pristine	Quartz filters, Illumina sequencing	*Bacillus, Eurotiomycetes, Malvales, Dothideomycete, Agaricales, Zygomycetes*	This study

A list of bacterial cultures isolated in this study, along with information on their Gram-nature, colony characteristics, pathogenic nature, GenBank accession numbers and phylogenetic tree for 16S rRNA gene sequences, is presented in Table 3 and Figure 5. Lanzhou is found to be predominant with *Bacillus* during the study period of May 2019. Bacteria such as *Bacillus halotolerans*; ATCC 25096, *Bacillus atrophaeus*; JCM 9070, *Bacillus subtilis* subsp. *Spizizeni*; NRRL B-23,049 was more abundant followed by *Staphylococcus equorum* subsp. *Equorum*; ATCC 43,958 and *Erwinia gerundensis*; EM595. Most of the bacteria isolated in the present study were Gram-positive (more than 90%) except *Erwinia gerundensis*; EM595, which is Gram-negative and plant pathogen. However, the pathogenicity of other strains identified in Lanzhou was not specified yet. The pathogenic nature of species is categorized using the online tool Global Catalogue of Microorganisms (http://gcm.wfcc.info/) and ABIS Encyclopedia (http://www.tgw1916.net/ABIS/encyclopedia.html). Similarly, Lhasa and Qomolangma were also found to be predominant with strains of *Bacillus*. As shown in Table 3, all three regions share similar strains of bacteria. This suggests that wind direction and dust events may have played some role in microbial transfer and abundance in certain areas. Plant pathogen was predominant in all three study sites. However, one human pathogenic bacteria *Kocuria rosea*; DSM 20,447 was isolated in culture in the laboratory from the bioaerosol samples obtained from Lhasa.

Table 3. National Center for Biotechnological Information (NCBI) BLAST search results for each of the identified bacterial strains.

Sample Name	Strain	Matched Accession Number	Similarity%	Length (bp)	Pathogenesis	Gram Stain	Colony Characteristics	GC Content%
LZB4	*Erwinia gerundensis*	KJ004603.1	99.39	1331	Plant pathogen	Gram-negative, rod shaped	Yellowish, circular	56.12
LZB7	*Staphylococcus equorum*	MN229550.1	100	1393	Produce cheese and meat order, May inhibit Listeria's growth	Gram-positive cocci	Opaque, white entire margin	50.54
LZB8	*Bacillus halotolerans*	MK517597.1	99.93	1390	Unknown	Gram-positive bacteria, rod shaped	Opaque, smooth, creamy colored	54.82
LZB10	*Bacillus atrophaeus*	NR_024689.1	99.86	1410	Unknown	Gram-positive bacteria, rod shaped	Opaque, smooth, creamy colored	55.04
LZB11	*Bacillus subtilis*	NR_116187.1	99.86	1408	Unknown	Gram-positive bacteria, rod shaped	Dull surface, thick/opaque, creamy colored, wrinkled (sometimes)	54.9
LSB1	*Bacillus aryabhattai*	NR_115953.1	99.79	1415	Unknown	Gram-positive bacteria, rod shaped	Opaque, smooth, creamy colored	53.57
LSB3	*Kocuria rosea*	NR_044871.1	99.85	1370	Infections in immunocompromised patients	Gram-positive cocci	Pinkish, smooth, shiny, circular	57.23
LSB5	*Bacillus altitudinis*	NR_042337.1	100	1382	Plant soft-rot causing pathogen	Gram-positive bacteria, rod shaped	White, regular margin	54.99
ZFB1	*Bacillus aryabhattai*	MK860027.1	100	1398	Unknown	Gram-positive bacteria, rod shaped	Opaque, smooth, creamy colored	53.58
ZFB2	*Bacillus aryabhattai*	NR_115953.1	99.79	1414	Unknown	Gram-positive bacteria, rod shaped	Opaque, smooth, creamy colored	53.54

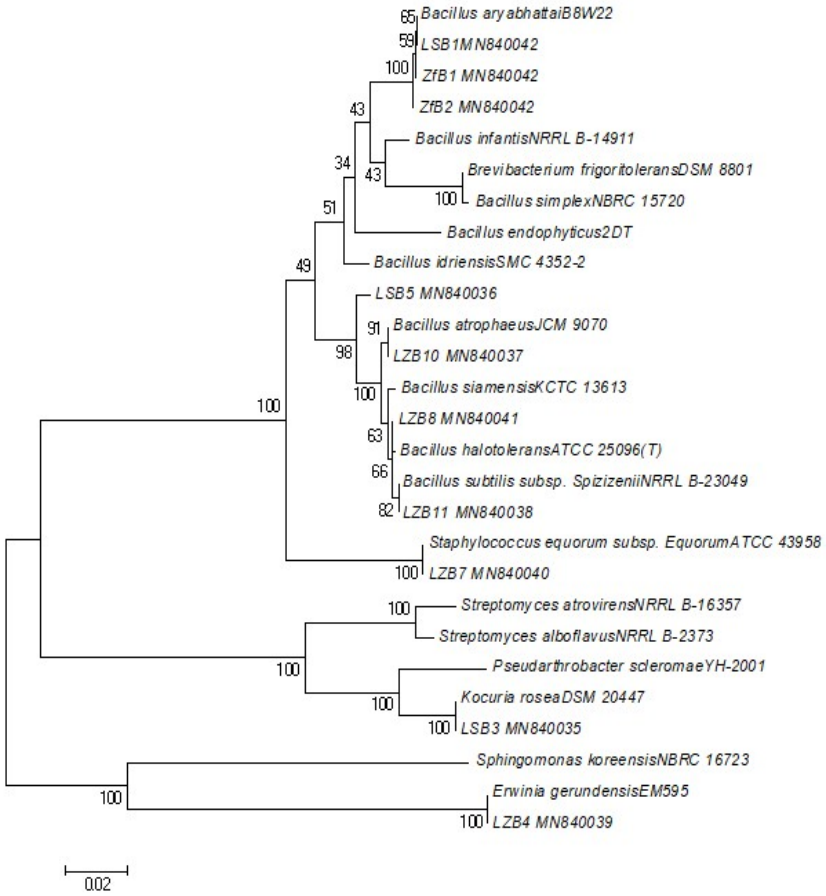

Figure 5. Phylogenetic analysis of the bacterial strain isolated from bioaerosol samples from three different regions. Based on related sequences of all the species were obtained from NCBI and aligned by MUSCLE through MEGA6. Evolutionary distances were computed using the maximum composite likelihood method. The obtained nucleotide sequences were deposited in the NCBI database and assigned with the accession number as MN840035-MN840042 for bacterial 16srRNA sequence.

Compared to bacterial culture isolation, more varieties of fungal cultures were isolated in all three sites. Based on the previous study, this could be because of the influence of dust storm, RH or other meteorological factors [25,56]. Culture of *Aspergillus flavus*, *Penicillium chrysogenum* isolate E20399, *Alternaria alternata* strain SCAU-F-91, *Chaetomium sp.* xz11 and *Coprinellus radians* strain F5 was isolated from Lanzhou aerosol in which most are plant pathogen and opportunistic human pathogen Table 4. However, Lhasa and Qomolangma are found to be enriched with and shares similar fungal isolates such as *Aspergillus niger*, *Rhizopus oryzae* isolate NDA02 and *Edgeworthia chrysantha* strain which also belong to plant pathogen and opportunistic human pathogen Table 4. Some isolated strains such as *Emericella rugulosa* isolate 211, *Coprinellus radians* strain F5 and *Edgeworthia chrysantha* strain F025 possess unknown pathogenic properties and need more study on its pathogenicity. A Maximum Likelihood tree generated using the ITS gene sequence dataset in MEGA is shown in Figure 6.

Table 4. NCBI BLAST search results for each of the identified fungal strains.

Sample Name	Strain	Matched Accession Number	Similarity%	Length (bp)	Pathogenesis	Colony Characteristics	GC Content (%)
LZF1	*Penicillium chrysogenum*	MK267448.1	100	563	Rare human pathogen, human allergen, source of antibiotics	Blue to blue–green conidia and the mold exudes a yellow pigment	57.02
LZF2	*Aspergillus flavus*	MG575511.1	100	564	Plant pathogen, opportunistic human and animal pathogen, causing aspergillosis in immunocompromised individuals	Powdery masses of yellow-green spores on the upper surface and reddish-gold on the lower surface	58.33
LZF3	*Aspergillus flavus*	MG991646.1	100	570	Plant pathogen, opportunistic human and animal pathogen, causing aspergillosis in immunocompromised individuals	Powdery masses of yellow-green spores on the upper surface and reddish-gold on the lower surface	58.07
LZF4	*Edgeworthia chrysantha*	MK961271.1	100	543	Not known. Possess anti-inflammatory and analgesic activity	Possesses pain brush type flowering	58.93
LZF5	*Aspergillus ustus*	MH865327.1	100	551	Human pathogen causing onychomycosis and otitis media, rarely found to cause endocarditis, pneumonia, disseminated disease, opportunistic pathogen in immunocompromised	Dull brown with a purplish to gray brown or dark brown colonies	58.08
LZF6	*Alternaria alternata*	MH865327.1	100	549	Opportunistic pathogen causing leaf spots, rots and blights on many plant parts	Black to olivaceous-black or greyish and are suede-like to floccose	46.27
LZF8	*Aspergillus sp.*	MH141246.1	99.82	550	Most commonly human, animal and plant pathogen, cause disease on many grain crops and some variants synthesize mycotoxins and aflatoxins	Powdery masses of yellow-green spores on the upper surface and yellowish on the lower surface	59.09
LZF9	*Chaetomium sp.*	KJ935022.1	99.82	544	Human allergens and opportunistic agents of ungual mycosis and neurological infections. Source of cellulose degrading enzymes	Cottony and white in color initially. Mature colonies become gray to olive in color	57.17
LZF10	*Coprinellus radians*	HQ380760.1	100	664	Unknown	Scattered yellowish-orange mat	49.4
LSF2	*Rhizopus oryzae*	MH865594.1	100	582	Opportunistic pathogen of humans causing mucormycosis. It is also used economically in the production of the enzymes, glucoamylase and lipase	Colonies are white initially, becoming brownish with age	40.55

Table 4. *Cont.*

Sample Name	Strain	Matched Accession Number	Similarity%	Length (bp)	Pathogenesis	Colony Characteristics	GC Content (%)
LSF3	*Rhizopus oryzae*	MH865576.1	100	602	Opportunistic pathogen of humans causing mucormycosis. It is also used economically in the production of the enzymes, glucoamylase and lipase	Colonies are white initially, becoming brownish with age	40.86
LSF6	*Emericella dentata*	MH032749.1	100	527	Unknown	Colonies are white and fluffy initially	59.58
LSF1	*Emericella rugulosa*	EU289912.1	99.82	541	Unknown	White to blackish sparse colony	59.7
LSF4	*Edgeworthia chrysantha*	MK806488.1	100	539	Not known. Possess anti-inflammatory and analgesic activity	Possesses pain brush type flowering	59.55
LSF8	*Aspergillus niger*	MK256745.1	100	573	Black mold of onions and ornamental plants, peanuts and grapes. Serious lung disease, aspergillosis in human. Produce important enzymes.	Granular to cottony, velvety or powdery; usually white at first and black at age.	58.46
ZFF2	*Aspergillus niger*	MK258199.1	100	577	Black mold of onions and ornamental plants, peanuts and grapes. Serious lung disease, aspergillosis in human. Produce important enzymes	Granular to cottony, velvety or powdery; usually white at first and black at age.	58.06
ZFF6	*Curvularia spicifera*	MK956807.1	100	543	Facultative pathogen or beneficial partner of many plant species	White to pinkish gray wooly colonies	46.96
ZFF11	*Rhizopus oryzae*	MK742815.1	100	501	Opportunistic pathogen of humans causing mucormycosis. It is also used economically in the production of the enzymes, glucoamylase and lipase	Colonies are white initially, becoming brownish with age	38.92
ZFF7.2	*Aspergillus tubingensis*	MF186869.1	100	578	Involved in food spoilage of fruits and wheat and industrial fermentation and a rare human pathogen.	Granular to cottony, velvety or powdery white-black colonies	57.96
ZFF1	*Aspergillus niger*	MK256745.1	100	572	Black mold of onions and ornamental plants, peanuts and grapes. Serious lung disease, aspergillosis in humans. Produce important enzymes	Granular to cottony, velvety or powdery; usually white at first and black at age.	58.57
ZFF4	*Edgeworthia chrysantha*	MK961271.1	100	540	Not known. Possess anti-inflammatory and analgesic activity	Possesses pain brush type flowering	59.44
ZFF5	*Aspergillus stellatus*	KU866665.1	99.82	541	Unknown	Colonies are initially while and later smooth orange to reddish brown	58.04

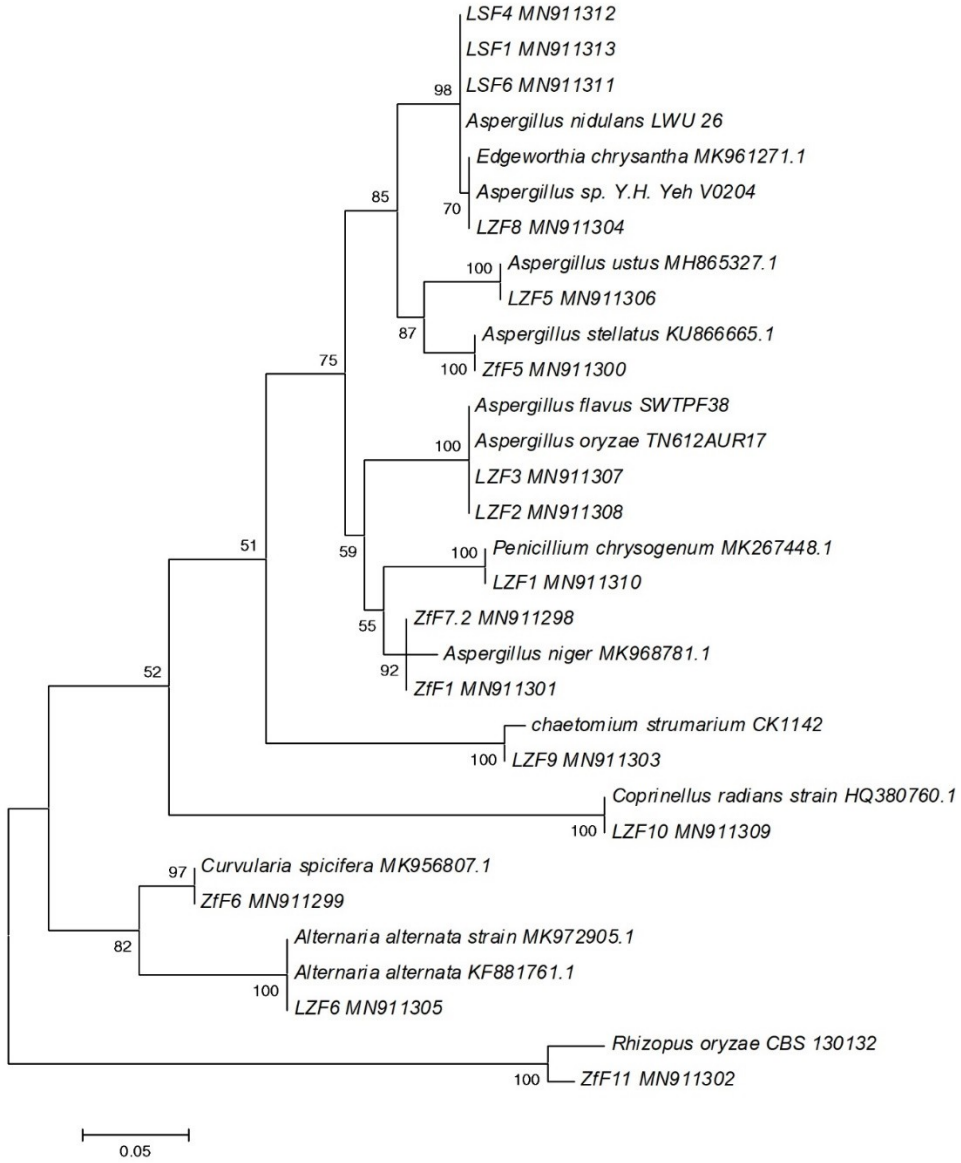

Figure 6. Phylogenetic analysis of the fungal strain isolated from bioaerosol samples from three different regions. Based on related sequences of all the species were obtained from NCBI and aligned by MUSCLE through MEGA6. Evolutionary distances were computed using the maximum composite likelihood method. The obtained nucleotide sequences were deposited in the NCBI database and assigned with the accession number as MN911298–MN911313 for fungal ITS sequence.

4. Discussion

The microbial abundance and structure present in ambient air have been observed several times in previous studies. When compared to previous analysis it can be seen that the level of airborne bacteria detected in this study is slightly different compared to other cities in the world such as Beijing, China (Bacteria: 5.8×10^3 CFU m^{-3}, Fungi: 7.2×10^3 CFU m^{-3}), Cincinnati, USA (Fungi: 3.8×10^3 CFU m^{-3}), Tijuana, Moscow (Bacteria: 1.7×10^3 CFU m^{-3}), Seoul, South Korea (Bacteria: 3×10^2 CFU m^{-3}, Fungi: 9×10^2 CFU m^{-3}) [57–60]. One of the reasons could be accredited to different meteorological and environmental conditions in different regions. In contrast, the previous studies by Li et al. (2017) and Wang et al., (2010) in Northwest region of China, such as Xian (Bacteria:1.9×10^3 CFU m^{-3}, Fungi: 1.7×10^3 CFU m^{-3}) and Dunhuang (Bacteria: 3.8×10^3 CFU m^{-3}) were comparable with the results from the present study [54,61]. Alike Xian and Dunhuang, the present study regions, are also located in Northwestern China, which is semi-arid or arid regions. Because of the dry condition and intense solar

radiation, the growth and survival of airborne microorganisms may be unfavorable [54,61]. On the other hand, Lanzhou and Lhasa being urban regions, the concentration of airborne microorganisms seem comparably higher, probably due to dust events occurred during May 2019 in Lanzhou. Previous studies have pointed out that dust can influence microbial growth [62], especially for fungal growth.

The inconsistency observed in previous studies about microbial communities in environmental samples provides some insight into the microbial physiological properties and their adaptation [63,64]. As for example, *Bacillus*, *Proteobacteria* and spores are most commonly found in the air; however, the community differs from location, season, altitude, etc. Yan et al. (2017) isolated the thermophilic sulfate-reducing non-spore forming bacterium *Desulfurispora*, from Beijing city of China [63]. In contrast, this strain was not isolated in this study period, which suggests the variation in strain dominance depending on geographical location. Several taxa were found to be related to specific regions, specific environment and haze levels [3,54,63,64]. However, unlike the presence of certain specific microbes in soil or rock or lake, the indication and discussion about specific and constant microbial communities in the air are not anywhere mentioned in previous studies. Hence, several factors may alter the airborne microbial community, and we can speculate variation in microbial composition in air acted upon at genetic (DNA, RNA or protein) molecular or metabolomics level.

Several previous studies have attempted to find the correlation between microbial concentration and environmental factors; nevertheless, the constant observation has not been recorded [17,52,54,63]. A study done by Yan et al. (2018) in Beijing air showed the correlated distributions of bacterial genera in relation to environmental factors are somehow in line with the current study and several other studies [63]. Bacterial genera, such as *Methylophilus*, *Ensifer*, *Meiothermus* and *Propionibacterium* were found to be positively correlated with temperature and RH at the lower concentrations of SO_2 and CO. However, genera such as, *Algoriphagus, Achromobacter* and *Brevibacillus* were observed to be negatively correlated with temperature and RH at the higher concentrations of SO2 and CO. On the other hand, Gao et al. (2016) observed negative correlation of bioaerosol concentration with season and temperature in the morning time, whereas positive correlation at the day time [52]. In this study, the wind rose plot and back-trajectories analysis suggested that the wind direction was mostly from the western side. Additionally, the air mass also arrived from southwest to two sites viz—Qomolangma and Lhasa and the western part of Gansu to Lanzhou, suggesting the westerlies wind could have an effect on the bioaerosols composition during the sampling period. The data presented in the study also showed that the bacterial and fungal loads showed significant correlations with RH and temperature. A significant positive correlation of fungal concentration with WS in Lanzhou was observed, whereas bacterial concentration in Lhasa and Qomolangma showed a statistically insignificant, but negative correlation. Past studies have demonstrated that the concentration of bioaerosols differs depending on the dominant season and climatic conditions because hydrodynamic and kinetic factors primarily direct the transport of bioaerosol and their fate is reliant on the chemical composition and the meteorological factors to which they are exposed [65]. Several studies have shown that seasonality in bacterial and viral infections [66]; suggesting the risk of contracting bacterial infections will be higher in the seasons with high concentrations of both indoor and outdoor bacteria. Reports from past studies have shown that spring and fall possess higher microbial concentration compared to other seasons. A study done by Frankel et al. in 2012, showed the seasonal pattern for indoor fungi, peaking from spring to summer and declining throughout fall to winter in urban areas of Australia and Central Europe [66–68]. Meanwhile, outdoor air from urban areas of Europe and the USA also revealed similar patterns in bioaerosol concentration. These findings support the approach of this study of choosing spring for sampling to develop a standard and reference point for analyzing bioaerosol in remote and urban areas of the TP region.

To our knowledge, this is the first study done on the three sites of Northwest China over the TP region. The above-discussed observation showed that urbanization seems to influence the diversity and richness of airborne microbial communities. The previous study done by Wei et al. (2015) observed a comparable concentration of viable bioaerosol particles among heavily polluted areas

and pristine regions [69]. This shows that both the meteorological factors, as well as the components of aerosol (TSP or PM) can act as carriers as well as supply the nutrition for microbial survival, growth and abundance. On the other hand, extreme environment, UV radiation, as well as highly concentrated environmental chemical or organic pollutants can also inactivate microbial functioning and abundance [63,70]. Thus, it would not be wrong to say that microbial survival and growth in the air is dependent on various environmental factors and is inconsistent and unpredictable. Similarly, there is another inevitable fact that microbes themselves possess unique physiology or may develop a feature that enables them to survive in the environment. For example, *Methylobacillus* and *Tumebacillus* are rich during hazy days. Moreover, small bacteria and spores can easily float in the air and increase in concentration [63,64,71]. Furthermore, it is considered that the variety of other volatile organic compounds, greenhouse gases as well as chemical composition have some role in the fungal and bacterial metabolic activities affecting their growth or survival [17], which has not been measured and considered in the study. The study also lacks the replicate sampling and long-term seasonal comparison. The other possible parameters that impact microbial composition and abundance have not been considered in the present study. Some other limitation of the study could be such as the surface area of fine and coarse particles which assists the microbial attachment and estimation of only the viable bioaerosols which are culturable. Because it has been found that only 1%–10% of microbial particles present in the air are cultivable in the laboratory provided specific growth conditions [17,62,72]. Hence, the study could be missing out on all other viable, but unculturable airborne microbes, which could be obtained by metagenomics analysis. Furthermore, only the microbial concentration (CFU/m^3) itself is inadequate and does not provide an actual concentration of bacteria and fungi present in the air in a given time. In addition to this, a long-term study and replicate sample will provide a more precise comparison and accurate interpretation of the result. More detailed investigations such as metagenomics and enzymatic analysis are essential and provide future direction to dig out more information on the relation of airborne microorganisms and environmental factors in the given study site.

Despite the restrictions of the culture-based method employed in the present study, the results of this campaign may still be useful in developing control or baseline data for bacterial and fungal loads and in predicting the occurrence of the microbial community including pathogenic bioaerosols.

In addition to this, the influence of environmental parameters on microbial loads and community composition could be drawn out. It should be noted that this preliminary assessment had created basic information on microbial population variation in the three sites in the TP region. Additionally, this study provides an easy, convenient and low-cost approach as well as the genetic basis for routine analysis of airborne microbes present in ambient air.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4433/11/5/527/s1, Table S1: Correlation matrix of the major parameters measured at Lanzhou site, China, Table S2: Correlation matrix of the major parameters measured at Lhasa site, China, Table S3: Correlation matrix of the major parameters measured at Qomolangma site, China.

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