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Profile and Characteristics of Culturable Airborne Bacteria in Hangzhou, Southeast of China

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ABSTRACT

Increased concentrations of airborne bacteria are known to be associated with decreased public health. As such, we evaluated the culturable concentration and distribution characteristics of airborne bacteria at four sampling sites in Hangzhou, southeast China. Results showed that the concentration of culturable bacteria in the air at selected sampling sites ranged from < 12 colony forming units (CFU) m^{-3} to 3259 CFU m^{-3} with a mean and a median of 292 and 201 CFU m^{-3}, respectively. We identified a total of 789 airborne bacterial isolates from multiple sampling sites and between different seasons, which distributed across 55 genera and 184 species of bacteria. *Micrococcus* (16.48%), *Bacillus* (13.94%), *Staphylococcus* (11.28%), *Kocuria* (11.28%), and *Pseudomonas* (4.94%) accounted for 58% of the total species and the dominant bacterial species were *Micrococcus luteus* (9.51%), *Kocuria roseus* (6.84%), *Bacillus megaterium* (4.56%), *Micrococcus roseus* (3.42%), and *Staphylococcus cohnii* (2.53%). Significant variation between sampling sites was observed with significantly higher bacterial concentrations detected at Yan'an Road Business Street (YRBS), followed by Tianmushan and Jiaogong Cross Road (TJCR) and Zhejiang Gongshang University Jiaogong Campus (ZJGSUJC), while the lowest concentrations were found at Breeze-ruffled Lotus at Quyuan Garden (BLQG) ($p < 0.05$). Moreover, seasonal variation of bacterial concentrations was observed across the different sampling sites: the highest bacterial concentrations in both YRBS and ZJGSUJC were found in autumn, followed by spring, and the lowest was found in winter ($p < 0.05$). No significant differences in seasonal patterns were found in BLQG ($p > 0.05$). Taken together these results provide a baseline for airborne culturable bacteria in southeast China, and will enable evaluation of the risks to human health from exposure to the atmosphere in the region.

Keywords: *Micrococcus*; *Bacillus*; Airborne bacteria; Concentration distribution; Composition characteristics.

INTRODUCTION

Bacteria are the most important biogenic aerosol particle found ubiquitously in the atmosphere (Jones and Harrison, 2004; Jaenicke, 2005). The concentration of bacterial aerosols correlates with the occurrence of human diseases and subsequent public health problems (Riley *et al.*, 1962, 1978; Fraser, 1980). Furthermore, elevated bacterial concentration in the air is closely associated with the increasing probability of food pollution, deterioration of cosmetics and medicine, and corrosion of metallic materials for infrastructure (Fang *et al.*, 2007). In addition, airborne bacteria are directly related to ecological processes in nature, including an important role in the natural matter cycle (Fang *et al.*, 2004). Recently, questions about potential interactions between airborne bacteria and the earth's biogeochemical systems have emerged (Deguillaume *et al.*, 2008). It has been reported that primary biological aerosols can affect atmospheric chemistry, and vice versa, through microbiological and chemical processes, subsequently impacting cloud formation and development (Ariya *et al.*, 2004; Christner *et al.*, 2008a, b; Burrows *et al.*, 2009; Pöschl *et al.*, 2010).

These adverse effects on human health and direct or indirect influences on ecosystem are driving forward research on airborne bacteria. However, such studies face significant challenges due to the broad diversity and tremendous variability in populations of airborne bacteria owing to different sources of origin, seasonal effects, local climate differences, weather patterns, local human activities, and local wind currents (Shaffer and Lighthart, 1997). In the past decades, many studies on the composition and distribution

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of airborne bacteria emerged around the world (Bovallius *et al.*, 1978; Mancinelli and Shulls, 1978; Jones and Cookson, 1983; Lighthart and Kin, 1989; Lighthart and Shaffer, 1995; Lighthart, 1997; Seino *et al.*, 2005; Abdel Hameed, *et al.*, 2009; Houdt *et al.*, 2009; Lee *et al.*, 2010; Wang *et al.*, 2010; Yassin and Almouqatea, 2010; Nasir *et al.*, 2012). These investigations significantly enriched our baseline knowledge of the normal abundance, distribution and composition of bacteria in the atmosphere, and which has supported many applications related to public health and international security. Indeed, it is necessary to collect detailed information about airborne bacteria from different environments with typical characteristics.

Since there is still little known about the characteristics, concentrations, and distribution of airborne bacteria in southeast China, we chose a typical tourist city, Hangzhou, as a model to measure the airborne culturable bacteria in this area. Hangzhou, the capital and largest city of Zhejiang Province in China, has a subtropical monsoon climate, and is warm in the winter and hot in the summer with four distinctive seasons and abundant precipitation. Impressively, Hangzhou has one of the most popular attractions in southeast China, West Lake, and is also regarded as one of the most desirable cities in China to live. The West Lake Cultural Landscape of Hangzhou has been inscribed on the World Heritage List by the United Nations Educational, Scientific and Cultural Organization (UNESCO) on June 24, 2011. In recent years, the number of tourists in Hangzhou has consistently increased with a total number of both Chinese and foreign tourists of 85.67 million in 2012. The present study was undertaken to assess the ambient culturable airborne bacteria concentration and composition in a typical tourist city in southeast China. The main objective of the study was to describe the diversity and concentration variation pattern of airborne culturable bacteria in Hangzhou both systemically and in great detail.

MATERIALS AND METHODS

Description of Sampling Sites

Four typical sampling sites were selected for this study based on their urban function (Fang *et al.*, 2007): (1) Tianmushan and Jiaogong Cross Road (TJCR), a heavily trafficked intersection located in Xihu district about 3 km from the city center; (2) Zhejiang Gongshang University Jiaogong Campus (ZJGSUJC), a cultural and educational area situated in Xihu district about 4 km from the city center; (3) Yan'an Road Business Street (YRBS), a commercial area and business district located at the center of Hangzhou city and in Xiacheng district; and (4) Breeze-ruffled Lotus at Quyuan Garden (BLQG), a scenic tourist area situated in Xihu district near West Lake, and about 5 km from the city center. Detailed information about these selected sites can be found in Table 1.

Sampling Methods and Strategy

We utilized an FA-1 sampler (imitated Andersen sampler, fabricated by the Applied Technical Institute of Liaoyang, China) for the collection of culturable airborne bacteria (Fang *et al.*, 2007). Each stage of the airborne bacterial sampling included a plate with 400 holes of uniform diameter through which air was drawn at 28.3 L min^{-1} before coming into contact with nutrient agar-filled petri dishes. Airborne particles were separated into six fractions; the aerodynamic cut-size diameters of 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 0.65–1.1μm (stage 6), respectively. FA-1 sampler was sterilized in a hot air oven at 180°C for 2 h before each 24 h measurement and subsequently washed with 5% bleach and 70% ethanol solution at the sampling site prior to collection.

Bacterial sampling in the air was conducted at four sampling sites throughout Hangzhou from Jul 2011 to Jun 2012. Sampling devices were operated at a sampling flow rate of 28.3 L min^{-1}, which was maintained with a platform at the height of approximately 1.5 m. Air samples were collected for 3 min in triplicate, three times daily (09:00, 13:00, and 17:00 hours) for three consecutive days of each month of the year. For each air sampling, the FA-1 sampler was loaded with 9.0 cm petri dishes containing nutrient agar (3 g beef extract, 10 g peptone, 5 g sodium chloride, 15 g agar, 1000 mL distilled water, pH 7.2). Exposed culture dishes were incubated for 48 h at 37°C.

Enumeration of Bacteria

After incubation, the colonies were counted, and the

Table 1. Detail information of the four selected sampling sites in Hangzhou.

Sampling sites	Functional type	Architecture type	Vehicle and personnel flow	Vegetation coverage
TJCR	Heavy traffic intersection	High and low office buildings and hotel around, main traffic road	With about 180 time min^{-1} flow of vehicles, and about 30 time min^{-1} flow of personnel	Less than 5 percent
ZJGSUJC	Cultural and educational area	Experimental buildings, classrooms, student dormitory and office buildings around	With about few flow of vehicle and about 10 time min^{-1} flow of personnel, and about 100 time min^{-1} flow of personnel off class	About 50 percent
YRBS	Commercial area and business district	Mall and many shopping buildings around	With 60 time min^{-1} flow of vehicles, and 80 time min^{-1} flow of personnel	Less than 5 percent
BLQG	Scenic tourist area	No buildings around	With few flow of vehicle and personnel	More than 95 percent

concentration of the samples was expressed as CFU per cubic meter of air (CFU m^{-3}). However, since superposition is unavoidable when the microbial particles impact the same spot through the same sieve pore, the colonies collected were recalculated using Macher's method (Macher, 1989; Fang *et al.*, 2007). Bacterial concentration was recorded as < 12 if total colonies collected with the sampler was less than one.

Identification of Bacteria

In this study, approximately 789 airborne bacteria were identified based on 16S rDNA sequence. The airborne bacterial colonies were isolated from different sampling sites (TJCR-195, ZJGSUJC-190, YRBS-206, and BLQG-198) in Hangzhou and from different seasons (spring-202, summer-202, autumn-187, winter-198) in a year. A representative number of bacterial colonies were selected randomly from the sampler plates to quantitatively estimate the kinds of bacteria. Approximately 5% of the colonies were selected, with no less than 6 colonies and no more than 10 due to sampling constraints.

Selected bacterial isolates were further identified as described below. Each pure isolate was homogenized in a liquid culture medium and then DNA was extracted using the CATB method (Möller *et al.*, 1992). The 16S rRNA gene was amplified using the following universal primer set synthesized by Shanghai Majorbio Bio-technology Company: 27F: 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 1492R: 5′-GGT TAC CTT GTT ACG ACT T-3′. The reaction mixture (50 μL) consisted of 0.3 μL Taq polymerase, 2 μL dNTP, 5 μL 10 × PCR buffer, 2 μL each primer, 1.0 μL (ca. 10 ng DNA) template, and 37.7 μL ddH_2O. The amplification program was as follows: initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and the final extension for 10 min at 72°C. The PCR products were purified and then detected by electrophoresis on a 1% agarose gel. The sequences were obtained using primer 27F by Shanghai Majorbio Bio-technology Company, and approximate 800 bp DNA sequence was utilized for the BLAST program of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/Blast.cgi). Sequences showing the highest similarity to those of the clones were extracted from GenBank, and 97%–100% similarity was used to assign a sequence to a bacterial species.

Statistical Analysis

All experimental data were analyzed with Excel 2010 and SPSS Version 19.0 (SPSS. Inc., Standard Version). The data for airborne bacterial concentration were normally distributed, and bacterial concentrations (including mean, median, and geometric mean concentration) were calculated by SPSS Version 19.0. One-way analysis of variance (ANOVA) was used to compare different sampling sites and sampling times, and the post hoc tests of all data analysis were carried out with the methods of Tukey and Duncan by SPSS Version 19.0.

RESULTS

Bacterial Concentration and Variation

Overall Bacterial Concentration

We surveyed the bacteria concentrations on our cultures from the four sampling sites from Jul 2011 to Jun 2012. Bacterial concentrations varied greatly at different sampling sites across Hangzhou, ranging from < 12 CFU m^{-3} to 3259 CFU m^{-3}. The mean and median bacterial concentrations were approximately 292 CFU m^{-3} and 201 CFU m^{-3} (Table 2).

Spatial Variations of Bacterial Concentration

Bacterial concentrations from the different sampling sites are demonstrated in Table 2. Significantly higher bacterial concentrations were measured in YRBS, followed by TJCR and ZJGSUJC, and the lowest concentrations were found in BLQG ($p < 0.05$). No significant differences in bacterial concentrations were detected between TJCR and ZJGSUJC ($p > 0.05$). The mean concentrations are as follows: YRBS (455 CFU m^{-3}), TJCR (277 CFU m^{-3}), ZJGSUJC (237 CFU m^{-3}), and BLQG (197 CFU m^{-3}).

Temporal Variations of Bacterial Concentration

Seasonal Variation of Bacterial Concentration

The total bacterial concentration from all four sites was highest during the autumn (376 CFU m^{-3}), followed by spring (313 CFU m^{-3}) and summer (273 CFU m^{-3}), and as expected was at the lowest during the winter (205 CFU m^{-3}) ($p < 0.05$). Individually, the highest concentrations from YRBS and ZJGSUJC were also observed in the autumn, followed by spring, and was the lowest during the winter ($p < 0.05$). Similarly, the lowest bacterial level was found during the winter in TJCR, however we did not observe a significant difference in bacterial concentration between summer, autumn, and spring months at this location ($p > 0.05$). No significant variations in bacterial concentrations were found among seasons in BLQG ($p > 0.05$) (Fig. 1).

Monthly Variation of Bacterial Concentration

The highest bacterial concentrations of all four sites

Table 2. Concentration background (average in a year) of airborne culturable bacteria at different sampling sites in Hangzhou (CFU m^{-3}).

Sampling sites	Minimum	Maximum	Mean	Median	Geo-mean
TJCR	< 12	3253	277 ± 33b	201	-
ZJGSUJC	< 12	2569	237 ± 28b	178	-
YRBS	12	3041	455 ± 35a	410	367
BLQG	< 12	2397	197 ± 31c	118	-
Total	< 12	3253	292 ± 33	201	-

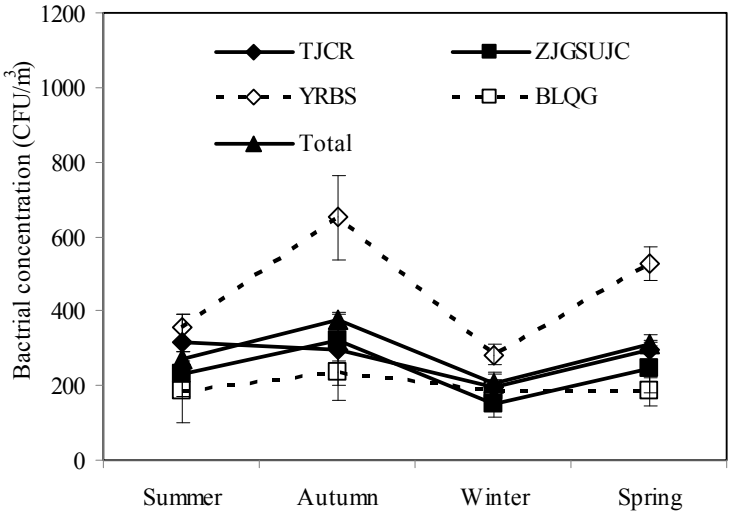

Fig. 1. Seasonal variation of airborne bacterial concentration at different sampling sites in Hangzhou (n = 27, 95% confidence interval).

combined were observed in Jun (475 CFU m^{-3}), Oct (425 CFU m^{-3}), and Sep (386 CFU m^{-3}), while the lowest concentrations were in Jan (136 CFU m^{-3}), Jul (160 CFU m^{-3}), and Aug (183 CFU m^{-3}). Analyzed individually, the bacterial concentrations within YRBS were highest from Sep to Nov and from Mar to Jun as compared withany other months during the year ($p < 0.05$), the highest concentration was observed in Oct (887 CFU m^{-3}) and the lowest during Jan (197 CFU m^{-3}). For the ZJGSUJC site, higher concentrations were found during Jun (410 CFU m^{-3}) and Sep (369 CFU m^{-3}), while the lowest concentrations were in Jul (74 CFU m^{-3}) and Jan (97 CFU m^{-3}). Within TJCR, the bacterial concentrations during Jun (590 CFU m^{-3}) and Mar (411 CFU m^{-3}) were the highest and Jan (95 CFU m^{-3}) and Aug (121 CFU m^{-3}) were the lowest. Finally, higher bacterial concentrations were detected in BLQG during Jun (450 CFU m^{-3}) and Oct (327 CFU m^{-3}) and were lowest during Jul (38 CFU m^{-3}) and Aug (63 CFU m^{-3}) (Fig. 2).

Diurnal Variation of Bacterial Concentration

In total, significantly higher bacterial concentrations were recorded at 9:00 and 17:00 as compared to 13:00 ($p < 0.05$). However, in BLQG we observed the lowest bacterial concentrations at 9:00, while each of the other sites (TJCR, ZJGSUJC, and YRBS) was lowest at 13:00 (Fig. 3).

Bacterial Groups and Variation Characterization

Overall Biodiversity of Culturable Bacteria

The percentage of gram positive bacteria, accounting for 88.2% of the total, was significantly higher than that of gram negative bacteria in the air of Hangzhou ($p < 0.001$) and the percentage of cocci was higher than bacilli (Table 3). A total

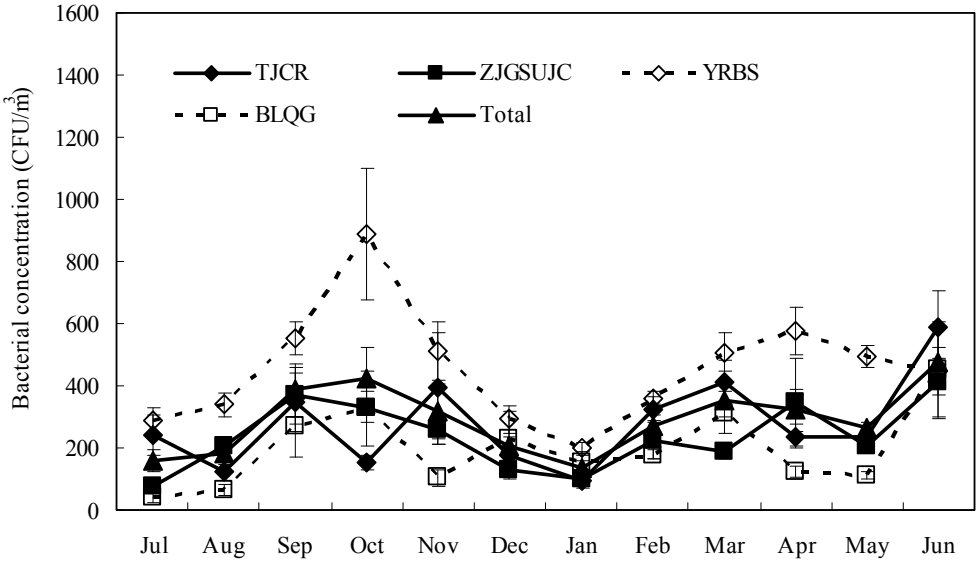

Fig. 2. Monthly variation of airborne bacterial concentration at different sampling sites in Hangzhou (n = 9, 95% confidence interval).

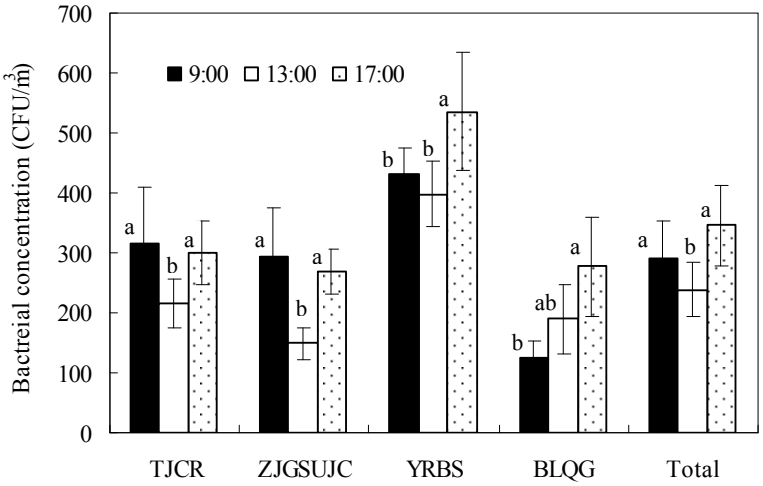

Fig. 3. Diurnal variation of airborne bacterial concentration in different sampling sites in Hangzhou (n = 36, 95% confidence interval).

Table 3. Type composition of airborne culturable bacteria at different sampling sites in Hangzhou.

	Gram positive (%)		Gram negative (%)
	Cocci	Rods	
Total	49.7 ± 4.7a	38.5 ± 3.0b	11.8 ± 1.9c
TJCR	45.1 ± 3.8a	42.6 ± 3.1a	12.3 ± 1.3b
ZJGSUJC	51.0 ± 2.0a	39.5 ± 2.7b	9.5 ± 1.6c
YRBS	55.8 ± 3.0a	35.4 ± 2.3b	8.8 ± 1.3c
BLQG	47.0 ± 2.3a	40.4 ± 2.0b	12.6 ± 2.1c

of 789 bacterial isolates belonging to 55 genera and 184 species were identified from the four sampling sites (Table 4). The most common bacteria populations were as follows: *Micrococcus* (16.48%), *Bacillus* (13.94%), *Staphylococcus* (11.28%), *Kocuria* (11.28%), and *Pseudomonas* (4.94%); these species accounted for approximately 58% of the total culturable airborne bacteria collected. The most common bacterial species were *Micrococcus luteus* (9.51%), *Kocuria roseus* (6.84%), *Bacillus megaterium* (4.56%), *Micrococcus roseus* (3.42%), and *Staphylococcus cohnii* (2.53%).

Biodiversity of Culturable Bacteria at Different Sampling Sites

We detected 35 bacterial groups, including 89 different species, from a total of 195 colonies in TJCR (Table 5). The most common bacteria groups were *Micrococcus*, *Bacillus*, *Kocuria*, *Staphylococcus*, and *Brevibacterium*, and the majority of the bacterial species were *Micrococcus luteus* (9.74%), *Kocuria rosea* (6.67%), and *Bacillus megaterium* (5.13%). In the ZJGSUJC site, 33 bacterial genera and 82 species were isolated from 190 selected colonies. *Micrococcus*, *Bacillus*, *Staphylococcus*, *Kocuria*, and *Brevibacterium* were dominant; the prevalent species were *Micrococcus luteus* (12.11%), *Kocuria rosea* (5.79%), and *Bacillus megaterium* (4.21%). For the YRBS samplingsite, we discovered 38 bacterial groups across 99 species isolated from 206 colonies. *Micrococcus*, *Kocuria*, *Bacillus*, *Staphylococcus*, and *Janibacter* were observed as the prevalent groups while *Micrococcus luteus* (9.22%), *Kocuria rosea* (8.25%), and *Micrococcus roseus* (5.34%) were the dominant species. Finally, 29 bacteria groups and 79 species were identified from 198 selected colonies in BLQG. The most common bacterial groups were *Bacillus*, *Micrococcus*, *Staphylococcus*, *Kocuria*, and *Pseudomonas*, and the dominant species were *Micrococcus luteus* (7.07%), *Kocuria rosea* (6.57%), and *Bacillus megaterium* (5.05%). These results indicated that the number of bacterial species was higher in YRBS, followed by TJCR and ZJGSUJC, and lower in BLQG ($p < 0.05$). Additionally, the percentage of *Bacillus* and *Pseudomonas* was higher in BLQG, and lowers in YRBS, while highest percentage of *Kocuria* and *Micrococcus* was detected in YRBS, and no significant difference of *Staphylococcus* percentage was found among the four sampling points.

Biodiversity of Culturable Bacteria at Different Seasons

We identified a total of 97 species across 36 genera of airborne bacteria from the selected 202 bacterial isolates during the spring at four sampling sites. The most common bacterial genus was found to be *Bacillus*, *Staphylococcus*, *Micrococcus*, *Pseudomonas*, *Kocuria*. During the summer, we isolated 202 bacterial colonies from a total of 91 species and 36 genera. The most common bacteria groups were *Micrococcus*, *Bacillus*, *Kocuria*, *Staphylococcus*, and *Corynebacterium*. During the autumn, a total 187 bacterial isolates belonging to 97 species and 40 genera were identified; the dominant bacteria were *Micrococcus*, *Staphylococcus*, *Kocuria*, *Bacillus*, and *Microbacterium*. Finally, 198 bacterial colonies were isolated during the winter and identified as

Table 4. Basic bacterial species in the air in Hangzhou.

Bacterial groups	Percentage (%)	Bacterial groups	Percentage (%)
Achromobacter cholinophagum	0.38	*Kocuria turfanensis*	0.13
Achromobacter spanius	0.13	*Kytococcus sedentarius*	0.51
Acinetobacter calcoaceticu	0.13	*Leuconostoc gelidum*	0.13
Acinetobacter johnsonii	0.25	*Luteococcus iaponicus*	0.13
Acinetobacter lwoffii	0.51	*Luteococcus sanguinis*	0.13
Actinomyces canis	0.13	*Lysinibacillus xylanilyticus*	0.13
Actinomyces hyovaginalis	0.13	*Macrococcus bovicus*	0.13
Actinomyces odontolyticus	0.13	*Macrococcus carouselicus*	0.38
Aerococcus urinae	0.13	*Macrococcus caseolyticus*	0.63
Aerococcus viridans	0.51	*Mcirobacterium oleivorans*	0.13
Aeromicrobium kwangyangensis	0.25	*Microbacterium arborescens*	0.25
Aeromonas hydrophilla	0.38	*Microbacterium chocolatum*	0.13
Aeromonas ichthiosmia	0.38	*Microbacterium esteraromaticum*	0.13
Aeromonas salmonicida	0.25	*Microbacterium flavescene*	0.38
Aeromonas veronii	0.25	*Microbacterium imperiale*	0.25
Agrobacterium larrymoorei	0.25	*Microbacterium laevaniformans*	0.25
Agrobacterium tumefaciens	0.51	*Microbacterium oleivorans*	0.25
Agrococcus jenensis	0.13	*Microbacterium pumilum*	0.25
Agromyces aurantiacus	0.13	*Microbacterium saperdae*	0.51
Arthrobacter cumminsii	1.01	*Microbacterium schleiferi*	0.13
Arthrobacter histidinolovorans	0.51	*Microbacterium testaceum*	0.13
Arthrobacter oxydans	0.25	*Microbacterium thalassium*	0.25
Arthrobacter woluwensis	0.25	*Micrococcus diversus*	1.52
Bacillus amyloliquefaciens	2.41	*Micrococcus luteus*	9.51
Bacillus aquimaris	0.25	*Micrococcus lylae*	1.52
Bacillus badius	1.39	*Micrococcus roseus*	3.42
Bacillus cereus	2.15	*Micrococcus terreus*	0.51
Bacillus marisflavi	0.13	*Paenibacillus campinasensis*	0.38
Bacillus maroccanus	0.76	*Paracoccus sp.*	0.13
Bacillus megaterium	4.56	*Pasteurella bettyae*	0.13
Bacillus mycoides	0.51	*Pasteurella multocida*	0.25
Bacillus nealsonii	0.13	*Pasteurella pneumotropica*	0.38
Bacillus pumilus	0.25	*Pediococcus acidilactici*	0.38
Bacillus subtilis	1.27	*Pediococcus pentosaceus*	0.38
Bacillus thioparans	0.13	*Pediococcus urinaeequi*	0.13
Blastococcus aggregatus	0.25	*Phyllobacterium rubiacearum*	0.63
Brachybacterium arcticum	0.38	*Planomicrobium glaciei*	0.51
Brachybacterium conglomeratum	0.13	*Pseudomonas aeruginosa*	0.13
Brachybacterium faecium	0.38	*Pseudomonas alcaligenes*	0.25
Brachybacterium nesterenkovii	0.38	*Pseudomonas bathycetes*	0.25
Brachybacterium sacelli	0.25	*Pseudomonas fluorescens*	0.51
Brevibacterium casei	1.27	*Pseudomonas fulva*	0.63
Brevibacterium epidermidis	0.13	*Pseudomonas geniculata*	0.13
Brevibacterium liquefaciens	0.38	*Pseudomonas glumae*	0.76
Brevibacterium lutescens	0.13	*Pseudomonas koreensis*	0.13
Brevibacterium massiliense	0.13	*Pseudomonas maculicola*	0.25
Brevibacterium mcbrellneri	0.13	*Pseudomonas marginalis*	0.38
Brevibacterium otitidis	0.76	*Pseudomonas mosselii*	0.13
Brevibacterium pityocampae	0.76	*Pseudomonas putida*	0.38
Brevundimonas diminuta	0.51	*Pseudomonas spinosa*	0.13
Brevundimonas vesicularis	0.38	*Pseudomonas syringae*	0.51
Cellulosimicrobium cellulans	0.25	*Pseudomonas tolaassi*	0.38
Chryseobacterium aquaticum	0.38	*Rhodococcus australis*	0.51
Citricoccus sp.	0.13	*Rhodococcus coprophilus*	0.25
Clavibacter agropyri	0.25	*Rhodococcus corynebacterioides*	1.14
Clavibacter michiganensis	0.25	*Rhodococcus erythropolis*	0.13

Table 4. (*continued*).

Bacterial groups	Percentage (%)	Bacterial groups	Percentage (%)
Corynebacterium accolens	1.01	*Rhodococcus globerulus*	0.38
Corynebacterium afermentans	0.25	*Rhodococcus kroppenstedtii*	0.25
Corynebacterium cystitidis	0.38	*Rhodococcus rhodochrous*	0.13
Corynebacterium mastitidis	0.51	*Rhodopseudomonas palustris*	0.25
Corynebacterium nitrilophilus	0.51	*Roseomonas aestuarii*	0.13
Corynebacterium urealyticum	0.25	*Roseomonas genomospecies*	0.25
Corynebacterium variabile	0.25	*Staphylococcus arlettae*	0.89
Corynebacterium vitaeruminis	0.51	*Staphylococcus aureus*	0.63
Corynebacterium xerosis	0.63	*Staphylococcus capitis*	0.63
Curtobacterium albidum	0.89	*Staphylococcus caprae*	0.13
Curtobacterium citreum	0.25	*Staphylococcus carnosus*	0.63
Curtobacterium flaccumfaciens	0.13	*Staphylococcus chromogenes*	0.25
Curtobacterium pusillum	0.89	*Staphylococcus cohnii*	2.53
Deinococcus grandis	0.25	*Staphylococcus delphini*	0.38
Deinococcus proteolyticus	0.13	*Staphylococcus epidermidis*	0.25
Dermabacter hominis	0.38	*Staphylococcus gallinarum*	0.13
Dermacoccus profundi	0.25	*Staphylococcus haemolyticus*	0.63
Dietzia maris	0.38	*Staphylococcus hominis*	1.27
Dietzia schimae	0.76	*Staphylococcus kloosii*	0.13
Enterobacter cowani	0.51	*Staphylococcus lentus*	0.38
Escherichia vulneris	0.13	*Staphylococcus pasteuri*	0.63
Exiguobacterium homiense	0.38	*Staphylococcus pulvererilvitulinus*	0.13
Exiguobacterium indicum	0.25	*Staphylococcus saprophyticus*	0.38
Exiguobacterium mexicanum	0.51	*Staphylococcus schleiferi*	0.13
Flavobacterium ferrugineum	0.38	*Staphylococcus sciuri*	0.63
Gordonia alkanivorans	0.13	*Staphylococcus warneri*	0.51
Janibacter marinus	0.38	*Stenotrophomonas maltophilia*	0.25
Janibacter melonis	1.77	*Streptococcus sanguinis*	0.51
Janibacter terrae	0.13	*Streptococcus suis*	0.25
Kocuria camiphila	0.51	*Streptococcus thoraltensis*	0.38
Kocuria gwangalliensis	0.13	*Vagococcus fluvialis*	0.13
Kocuria halotolerans	0.63	*Vagococcus lutrae*	0.13
Kocuria marina	0.51	*Vagococcus salmoninarum*	0.13
Kocuria palustris	0.25	*Vibrio carcariae*	0.13
Kocuria rhizophila	2.28	*Vibrio harveyi*	0.13
Kocuria rosea	6.84	*Xanthomonas campestris*	0.38

86 species belonging to 32 genera. The most common bacterial groups were *Micrococcus*, *Staphylococcus*, *Kocuria*, *Bacillus*, and *Brevibacterium* (Table 6). In addition, higher percentage of *Bacillus* was detected in the spring, and lowers in the autumn and winter. The *Micrococcus* percentage was highest in the summer, while highest *Pseudomonas* percentage was observed in the spring.

DISCUSSION

To understand the effect of airborne microbes on human health and to explore the role of microbes in the ecosystem, the first step is to characterize the microbial background in different environments. The current study presented the concentrations and compositions of airborne culturable bacteria in Hangzhou, southeast of China. Result showed that significantly higher bacterial concentration was detected in the areas of enriched human activity and high human traffic, and the lowest was found in the less populated areas. This result supports the model that human activities, personnel and vehicle flow, and other similar factors cause an increase in bacteria concentration and diversity throughout a city (Ju *et al.*, 2003; Fang *et al.*, 2007), while green areas with multiple trees, shrubs, and herbaceous plants show much lower bacterial concentration and diversity (Ju *et al.*, 2003; Chen *et al.*, 2008; Pan *et al.*, 2010). Meanwhile, our study also showed that the percentage of gram positive bacteria at ZJGSUJC and YRBS was much higher than that at the sampling sites of BLQG. It has been reported that gram positive bacteria (especially coccus) can be isolated from the skin, mucous membrane, and other parts of humans and animals. The high activity of personnel flow is thought to cause the release of gram positive bacteria into the air (Koneman *et al.*, 1995). Moreover, gram positive bacteria contributed to approximately 88.2% of the overall bacteria in the air in Hangzhou, since gram positive bacteria are much more resiliant than gram negative bacteria in the adverse external environmental conditions, such as poor nutrient

Table 5. Bacterial groups at different sampling sites in the air in Hangzhou.

Bacterial groups	TJCR (%)	ZJGSUJC (%)	YRBS (%)	BLQG (%)
Achromobacter	1.03	-	0.49	0.51
Acinetobacter	0.51	1.05	0.97	1.01
Aerococcus	-	1.05	0.49	1.01
Aeromonas	1.03	1.05	2.43	0.51
Agrobacterium	0.51	1.05	0.49	1.01
Arthrobacter	1.54	2.63	1.46	2.53
Bacillus	14.36	12.63	11.17	17.17
Brachybacterium	0.51	2.11	1.46	2.02
Brevibacterium	6.67	4.74	0.97	2.53
Brevundimonas	0.51	1.05	0.49	1.52
Chryseobacterium	0.51	0.53	0.49	-
Corynebacterium	5.13	4.74	3.40	4.04
Curtobacterium	2.56	3.68	-	2.53
Dermabacter	0.51	0.53	1.46	-
Dietzia	1.54	-	0.97	2.02
Janibacter	0.51	3.16	3.88	1.52
Kocuria	11.79	9.47	14.08	9.60
Kytococcus	0.51	1.05	0.49	-
Macrococcus	-	1.58	1.46	1.52
Microbacterium	2.56	2.63	3.40	3.54
Micrococcus	16.92	17.37	18.45	13.13
Pasteurella	1.03	1.05	0.97	-
Pediococcus	-	1.05	0.97	1.52
Pseudomonas	5.64	4.21	3.40	6.57
Rhodococcus	2.05	1.58	3.40	4.04
Staphylococcus	10.77	11.58	10.68	12.63
Streptococcus	1.03	1.05	1.46	1.01
Xanthomonas	0.51	0.53	0.49	-
Others	9.76	6.85	10.13	6.51

availability, desiccation, and strong solar radiation (Tong and Lighthart, 1997a, b).

We observed seasonal variations of bacterial concentration between the different sampling sites. Our results were in accordance with the studies of Bowers *et al.* (2012) which demonstrated that bacterial abundances varied by season with the highest concentrations in fall and spring. However, these results are different from our previous study at the Research Center for Eco- Environmental Sciences (RCEES) and Xizhimen (XZM) in Beijing, where we found much higher bacterial concentrations during autumn and summer months (Fang *et al.*, 2007). Hangzhou is located in southeast of China while Beijing is situated in the north of China. During the summer, extreme temperatures in Hangzhou far exceeding 39°C has the potential to significantly reduce bacterial concentrations in the air (Data not shown). Moreover, significantly higher bacterial concentrations were found at 9:00 and 17:00 than at 13:00 ($p < 0.05$). The major influencing factors included human activities, sunlight, temperature, the regular variations of atmospheric stability, and etc. The first sampling time (09:00) in a day was around the morning rush hour when both human activities and traffic were at the peak. Meanwhile, the atmosphere was appeared with reverse temperature and low radiation in the near ground. These conditions are ideal for increased bacterial concentrations. As time advanced from 09:00 to 13:00, the air temperature increased gradually, and the bacterial particles were vertically diffused and diluted in the lower layer. In addition, airborne bacteria were damaged by UV radiation with the gradual increase in radiation. At sunset, the atmosphere appeared with reverse temperature in the low layer again, which would restrain the vertical exchange of bacterial particles. Additionally, low radiation, and rush hour of person and vehicle in the road might lead to higher bacterial concentration at 17:00.

Interestingly, we noticed that the mean bacterial concentration was much lower in Hangzhou (292 CFU m^{-3}) than in Beijing (2217 CFU m^{-3}) (Fang *et al.*, 2007). Beijing has a continental monsoon climate with cold dry winters and arid windy springs, which leads to many days of sand and dusty weather throughout the year. As such, urban plants only flourish in summer. In contrast, Hangzhou's climate is subtropical and seldom ever has sandy or dusty weather, and urban plants grow very well even during the winter. Sand and dust near the ground is known to be one of the main sources of airborne bacteria (Polymenakou *et al.*, 2008; Chen *et al.*, 2010), while volatile secretions released by plants can disinfect bacteria in the air (Xie *et al.*, 1999). We hypothesize that these factors directly result in lower concentrations of airborne bacteria in Hangzhou as compared to Beijing. Meanwhile, the dominant airborne bacteria in Hangzhou differed slightly from published results. One of the prevalent

Table 6. Bacterial groups in the air in different seasons in Hangzhou.

Bacterial groups	spring (%)	summer (%)	autumn (%)	winter (%)
Achromobacter	0.50	0.99	0.53	0.51
Acinetobacter	0.50	1.49	1.07	-
Aerococcus	0.50	0.99	1.07	-
Aeromonas	1.49	1.98	0.53	1.01
Agrobacterium	0.99	0.50	1.07	0.51
Arthrobacter	0.99	2.97	1.60	2.53
Bacillus	18.32	15.84	10.70	10.61
Brachybacterium	0.99	0.50	1.07	3.54
Brevibacterium	3.47	3.47	2.67	5.56
Brevundimonas	1.49	0.99	0.53	0.51
Clavibacter	0.50	0.50	0.53	0.51
Corynebacterium	4.46	4.95	3.74	4.04
Curtobacterium	1.49	2.48	1.07	3.54
Deinococcus	-	0.50	0.53	0.51
Dietzia	-	0.99	2.67	1.01
Enterobacter	0.50	-	0.53	1.01
Exiguobacterium	0.99	0.99	0.53	2.02
Flavobacterium	0.50	0.50	-	0.51
Janibacter	3.96	0.99	2.14	2.02
Kocuria	8.91	11.88	11.76	12.63
Kytococcus	-	0.50	1.07	0.51
Macrococcus	0.50	0.99	1.60	1.52
Microbacterium	1.98	1.98	5.35	3.03
Micrococcus	12.87	21.29	16.04	15.66
Pasteurella	-	1.98	0.53	0.51
Pediococcus	1.49	0.50	1.07	0.51
Phyllobacterium	1.49	0.50	-	0.51
Pseudomonas	8.91	3.47	3.21	4.04
Rhodococcus	1.98	1.98	3.21	4.04
Staphylococcus	12.87	7.43	11.76	12.63
Streptococcus	-	1.49	1.60	1.52
Others	7.36%	4.39	10.22	2.94

bacteria in Beijing is known to be *Corynebacterium* (Fang *et al.*, 2007), which was replaced by *Kocuria* in Hangzhou. This difference is likely due to the differences in climatic conditions between these areas. The most common bacteria, *Micrococcus* and *Kocuria*, are able to produce colored (pink, yellow, orange, and red) pigment, which are thought to act as "sunscreen" for the organisms protecting them from UV radiation (Tong and Lighthart, 1997a, b). Moreover, *Bacilli* have the ability to form spores that facilitate in their resistance to harsh environmental conditions such as UV radiation, desiccation, lack of nutrients, and/or extreme temperatures (Mandal and Brandl, 2011). Taken together, it is thought that the metabolic capabilities of bacteria facilitate the predominant distribution and survival within the atmosphere of different outdoor environments.

In our study, the incubation temperature (36 ± 1°C) of airborne bacteria was designed according to the State Standard of China (GB/T 18204.3-2013), and 36 ± 1°C is the suitable incubation temperature for most bacteria at the atmosphere. Meanwhile, we have taken seriously consideration about the problems of studying only culturable airborne bacteria, since the vast majority of environmental bacteria are nonculturable even when viable (Wainwright *et al.*, 2004). Approximately, the fraction of airborne bacteria that can be detected by culture methods is typically less than 10% (Heidelberg *et al.*, 1997; Lighthart 1998). With the development of molecular biology technology, quantitative polymerase chain reaction (Q-PCR) followed by high- throughput DNA sequencing has been developed as an emerging methods in investgating airborne bacteria (Oppliger *et al.*, 2008; Hospodsky *et al.*, 2010; Cao *et al.*, 2014). Nevertheless, this method also presents obvious disadvantages. Q-PCR is unable to differentiate between viable and dead bacteria accurately. Efficient air sampling and isolation of high quality genomic DNA from air samples is challenging. To make a better comparison with our former studies, we chose the traditional culture method according to the State Standard of China (GB/T 18204.3-2013). We agree that culture studies followed up with Q-PCR and DNA sequencing to obtain quantitative results would be a better approach going forward.

Taken together our results provide an exposure database of airborne bacteria in the area of southeast of China. Furthermore, when compared to data from a previous study published from this lab, our results have begun to reveal regional differences in bacterial composition characteristics and concentration distributions between north and south China.

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