African Journal of Microbiology Research Vol. 6(3), pp. 665-673, 23 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1189
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Assessment of culturable airborne bacteria in a university campus in Hangzhou, Southeast of China

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Accepted 29 November, 2011

A systematic investigation on the varieties of culturable airborne bacteria component, concentration and size distribution was conducted in a university campus in Hangzhou, Southeast of China. Results obtained showed that Gram positive bacteria were much more than Gram negative bacteria, contributing to about 84 to 90% of the total number. 22 genera of Gram positive and 12 genera of Gram negative bacteria were identified. *Micrococcus*, *Bacillus, Staphylococcus*, and *Corynebacterium* were dominant in Gram positive bacteria, while *Pseudomonas* were most common in Gram negative bacteria, and these 5 dominant genus occupied about 60% of the total generally. The mean concentration of culturable bacteria in the university campus was 1224 CFU (Colony Forming Units) /m^3. Bacterial concentration in living area was highest (1572 CFU/m^3), while the concentration in office area was lowest (651 CFU/m^3), obviously lower than other areas in the campus (P < 0.01). With regard to the bacterial size distribution, there were no significances among different sampling areas and seasons. The percentage of the culturable bacteria was gradually increased from stage 1 (> 8.2 µm) to stage 5 (1.0 to 2.0 µm), and then was dramatically decreased at stage 6 (<1.0 µm) in the sampler. Moreover, airborne bacteria was mostly collected in stage 3 (3.0 to 6.0 µm), stage 4 (2.0 to 3.5 µm) and stage 5 (1.0 to 2.0 µm), and the highest proportions of culturable airborne bacteria were detected at stage 5.**

Key words: Airborne bacteria, *Micrococcus*, *Bacillus*, size distribution.

INTRODUCTION

Aerosol dispersal of pathogens such as airborne bacteria and fungi pose important health and ecological issues. Studies on atmospheric bacteria have been driven by the need to determine the concentration, species, source and/or their correlation factors of potential pathogens. Up to now, airborne bacteria have been predominantly approached in the context of their toxicity, allergies and general medical implications in specific indoor environments such as hospital premises (Miner et al., 2005), homes and offices (Gorny and Dutkiewicz, 2002; Tsai and Macher, 2005), animal houses (Zucker and Muller, 2002) and processing plants (Dutkiewicz et al., 2002). There is an emergent need for baseline information about the normal abundance, distribution and composition of bacteria in the atmosphere to support many applications related to public health and international security. Such studies face significant challenges, including the broad diversity of bacteria that can be carried into the air from soil and plant sources, and the tremendous variability (both locally and regionally) in microbial load and composition owing to seasonal effects, local climate, weather patterns, local human activities, and local wind currents (Lighthart, 1997). Thus, it is necessary to collect detailed information about airborne bacteria all over the world, both indoor

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Abbreviations: LA, living area; **OA,** office area; **DA,** dining area; **TA,** teaching area

and outdoor environments with typical characteristics to enrich the database. Many studies were carried out about the bacteria community in outdoor, indoor and even underground environments (Seino et al., 2005). However, little is known about the composition, concentration, and size distribution of airborne bacteria in Southeast of China. What's more, the university campus environment has recently attracted attention widely in China, since the Chinese government proposed expand university enrolment of professional and specialized graduates and develop world class universities in 1998. Enrolment in higher education increased rapidly year by year whereas the infrastructure construction couldn't keep up with the university expanding program very well, and the campus of many universities became relatively crowded.

Taken together, it is indispensable to survey both concentration variation pattern and composition of airborne bacteria systematically and extensively in these areas in China. The present study was undertaken to assess the ambient culturable airborne bacteria concentrations and community structure in the southeast of China. Here, we chose four sampling sites in different indoor environments in the campus of Zhejiang Gongshang University in Hangzhou city, southeast of China. The main objectives of the study were to describe the groups, concentration variations, and size distribution of airborne culturable bacteria in the campus.

MATERIALS AND METHODS

Site description

Hangzhou is the capital and largest city of Zhejiang Province in southeast of China with a registered population of about 8.7 million. It has a humid subtropical climate with four distinctive seasons, characterized by long, very hot, humid summers and short, chilly, cloudy and dry winters (with occasional snow). The average annual temperature is 16.5°C (61.7°F), ranging from 4.3°C (39.7°F) in January to 28.4°C (83.1°F) in July. The city receives an average annual rainfall of 1,450 millimetres (57.1 in) and is affected by the plum rains of the Asian monsoon in June. In the present study, four sampling sites located in the campus of Zhejiang Gongshang University in Hangzhou city were selected for the study of investigation on culturable airborne bacteria: (i) Living area (LA), a dormitory with the area of about 20 m^2 on the second floor of a building in Zhejiang Gongshang University. There were 6 graduate students living in it, and the indoor environment was not so clean. They do the sweeping about once a week. (ii) Dining area (DA), a canteen about 400 m^2 on the first floor of Xinlanyuan Building in Zhejiang Gongshang University. It can hold more than 300 people for dinner, and there are special barrels for plate and bowl leftover near the door. The indoor environment is pretty well, and they do the sweeping after each dinner. (iii) Teaching area (TA), an extremely large classroom that can hold more than 130 students in Zhejiang Gongshang University. The indoor environment is ordinary, not so clean or dirty. (χ) Office area (OA), a teacher office about 15 m^2 on the second floor in a building in Zhejiang Gongshang University.

There are 3 teachers Zhejiang Gongshang University working in it, and with 3 office tables and 2 bookcases. The indoor environment is very well, and the teachers sweep the floor and open the window everyday.

Sampling methods

A six-stage culturable FA-1 sampler (imitated Andersen sampler), made by the Applied Technical Institute of Liaoyang, China, was used to isolate culturable bacteria from the air in the university campus. Each stage includes a plate with 400 holes of uniform diameter through which air is drawn at 28.3 L/min to impact on Petri dishes containing agar media. Airborne particles were separated into six fractions, and the aerodynamic cut-size diameters in six stages were 7.0 µm (stage 1), 4.7 to 7.0 µm (stage 2), 3.3 to 4.7 µm (stage 3), 2.1 to 3.3 µm (stage 4), 1.1 to 2.1 µm (stage 5), and 0.65 to 1.1 µm (stage 6), respectively. In each sampling area, the sampler was mounted on 1.5 m above ground level with a platform. Sampling was conducted seasonally in October, 2009 (autumn), January, 2010 (winter), April, 2010 (spring), and July, 2010 (summer), respectively in a year. All of the samples were collected at 14:00 for 3 min in triplex, and continued for three consecutive days of each season. For each 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. Results were then expressed as colony forming units per cubic meter of air (CFU/m^3).

Sample analysis

Bacterial concentration determination

Colony forming units (CFU) on each plate were counted and concentration of samples was expressed as CFU per cubic meter of air (CFU/m^3). However, since the superposition is unavoidable when the microbial particles impact the same spot through the same sieve pore, the colonies collected was revised by Equation 1. CFU/m^3 was calculated by Equation 2:

$$P_r = N\left(\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \cdots + \frac{1}{N-r+1}\right) \quad (1)$$

$$C(CFU/m^3) = \frac{(P_1 + P_2 + P_3 + P_4 + P_5 + P_6) \times 1000}{t(\ \min) \times F(L/\min)} \quad (2)$$

In the equations, P_r is the revised colony in every stage (r is from 1 to 6); N is the number of sieve pore in every stage of the sampler; r is the sampling colony; C is airborne bacterial concentration; P_1, P_2, P_3, P_4, P_5, and P_6 is the revised colony in every stage in the sampler; t is the sampling time; F is the air flow rate of sampler during sampling.

Bacterial particle percentage determination at every stage in the sampler

The bacterial particle percentage at every stage was calculated by (3):

$$BP_r = \frac{P_r}{P_1 + P_2 + P_3 + P_4 + P_5 + P_6}$$

$$BP_r = \frac{P_r}{P_1 + P_2 + P_3 + P_4 + P_5 + P_6} \quad (3)$$

In the equation, BP_r is the bacterial particle percentage (r is from to 6); P_r is the revised colony in every stage (r is from 1 to 6); P_1, P_2, P_3, P_4, P_5, and P_6 is the revised colony in every stage in the sampler

Table 1. Percentage of Gram-positive and Gram-negative bacteria in four sampling sites in the university campus.

Sampling sites	Gram-positive		Gram-negative
	Cocci	Rods	
LA	52.8	34.5	12.7
DA	59.1	30.9	10.0
TA	51.8	32.5	15.7
OA	54.6	29.4	16.0

Bacterial identification

Bacterial isolates selected from the sampling sites were further identified using the molecular method as described below. Each pure isolate was homogenized described below in liquid culture medium and then DNA was extracted using CATB method (Möller et al., 1992).

The 16S rRNA gene was amplified using the following universal primer set: 27F: 5′-AGA PTT TGA TCC TGG CTCAG-3′ and 1542R: 5′-ACG GCT ACC TTG TTA CGA CT-3′. The reaction mixture (50 µL) consisted of 0.3 µL Taq polymerase, 2 µL dNTP, 5 µL10×PCR buffer, 2 µL each primer, and 1.0 µL (ca. 10 ng DNA) template. 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, and extension at 72°C for 30 s, and then 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 prime T7 by the Shanghai Majorbio Bio-technology Company, and were analyzed with the BLAST program of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/Blast.cgi). The sequences showing the highest similarity to those of the clones were extracted from GenBank.

Statistical analysis

All the experimental data were analyzed using SPSS Version 17.0 and the Figures were made by Microsoft Excel 2007. The multiple comparative analysis method of ANOVA and Duncan's test was used to assess the differences of concentration of airborne bacteria among the investigated sites and different seasons in the sampling year. The significant differences of airborne bacteria concentrations were analyzed by means of paired t-test.

RESULTS

Bacterial groups in the university campus

Total 533 bacterial colonies from the four sampling sites were isolated and identified in the present study. The percentage of Gram-positive bacteria, accounting for about 84 to 90%, was significantly higher than that of Gram-negative bacteria in the air in the university campus (**P < 0.01), and the percentage of cocci (55 to 60%) was remarkable higher than that of rods (29 to 35%) (**P < 0.01). In different sampling areas of the university campus, significantly higher percentage of Gram-positive bacteria was observed in LA and DA than in TA and OA (*P < 0.05). On contrary, the higher percentage of Gram-negative bacteria was recorded in TA and OA (*P < 0.05). In the group of Gram positive bacteria, highest cocci percentage was detected in DA, and lowest in TA, while highest rod percentage was recorded in LA, and lowest in OA (Table 1)

With regard to airborne bacterial groups, total 34 genera of culturable bacteria were detected from all sampling area in the university campus, and there were 22 genera of Gram-positive and 12 genera of Gram-negative bacteria, accounting for 64.7 and 35.3%, respectively (Table 2). As a whole, the dominant Gram-positive bacteria were *Micrococcus*, *Bacillus*, *Staphylococcus*, and *Corynebacterium* according to priority, and the main Gram-negative bacteria were *Pseudomonas* amongst all the bacterial genera from the bacterial percentage data. These five dominant culturable bacteria in the university campus contributed to about 60% of the total airborne bacteria. *Micrococcus*, accounting for about 22 to 29%, was the most dominant genus, and *Pseudomonas* occupied 2.0 to 5.0% of the total culturable airborne bacteria.

In different sampling area, 30 genera (134 bacterial colonies), including 20 genera of Gram-positive and 12 genera of Gram-negative bacteria in LA, 30 genera (140 bacterial colonies), including 22 genera of Gram-positive and 8 genera of Gram-negative bacteria in DA, 29 genera (134 bacterial colonies), including 19 genera of Gram-positive and 10 genera of Gram-negative bacteria in TA, and 27 genera (125 bacterial colonies), including 18 genera of Gram-positive and 9 genera of Gram-negative bacteria in OA were identified, respectively. The dominant bacterial genus were *Micrococcus*, *Bacillus*, *Staphylococcus*, *Corynebacterium*, *Microbacterium* and *Pseudomonas* according to priority in LA, and *Micrococcus* was the most dominant genus in DA, followed by *Bacillus*, *Staphylococcus*, *Corynebacterium*, *Curtobacterium* and *Pseudomonas*. In TA, *Micrococcus*, *Staphylococcus*, *Bacillus*, *Microbacterium*, *Corynebacterium*, *Pseudomonas*, and *Curtobacterium* was the dominant bacterial genera. The most common bacteria in OA were *Micrococcus*, *Bacillus*, *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, and *Microbacterium*. Generally, *Micrococcus* was the most dominant bacterial in the air in the university campus, and its percentage was about 23.9% in LA, 28.6% in DA, 26.9% in TA, and 22.4% in OA.

Table 2. Community composition of airborne bacteria in four sampling sites in the university campus (%).

Bacteria groups	Sampling sites			
	LA (%)	DA (%)	TA (%)	OA (%)
Gram positive	87.3	90.0	84.3	84.0
Acinobacterium	0.7	0.7	1.5	0.8
Aerococcus	1.5	1.4	0.7	1.6
Arthrobacter	1.5	0.7	1.5	2.4
Bacillus	17.9	14.3	11.9	17.6
Brachybacterium	2.2	2.9	1.5	1.6
Clavibacter	0.7	2.9	2.2	1.6
Corynebacterium	6.7	5.7	6.0	8.0
Curtobacterium	2.2	4.3	3.0	2.4
Deinococcus	0.7	0.7	0.7	0.8
Dermabacter	1.5	0.7	0.0	0.0
Exiguobacterium	1.5	2.1	0.7	1.6
Kurthia	0.7	0.7	0.0	0.0
Kytococcus	0.0	0.7	0.7	0.0
Leuconostoc	0.7	1.4	1.5	2.4
Macrococcus	0.	0.7	1.5	0.8
Microbacterium	3.0	3.6	1.5	3.2
Micrococcus	23.9	28.6	26.9	22.4
Paenibacillus	0.0	0.7	0.0	0.0
Pediococcus	0.7	0.7	2.2	1.6
Rhodococococcus	1.5	2.1	1.5	0.8
Staphylococcus	11.2	10.0	13.4	9.6
Streptococcus	2.2	1.4	0.7	0.8
No-identification	5.2	2.9	4.5	4.0
Gram negative	12.7	10.0	15.7	16.0
Achromobacter	0.7	0.0	0.7	0.8
Acinetobacter	1.5	1.4	1.5	1.6
Aeromonas	0.0	0.7	0.7	0.8
Brevundimonas	0.7	0.0	0.0	0.0
Escherichia	0.7	0.7	0.0	0.0
Flavobacterium	0.7	0.7	1.5	1.6
Pantoea	0.7	1.4	0.7	0.8
Pasteurella	0.7	0.0	1.5	0.8
Phyllobacterium	0.0	0.7	0.7	0.0
Pseudomonas	3.0	2.1	3.7	4.8
Vibrio	0.7	0.7	0.7	1.6
Xanthomonas	0.7	0.0	0.7	0.8
No-identification	2.2	1.4	3.0	2.4

Concentration distribution of culturable airborne bacteria in the university campus

Spatial variation pattern of culturable airborne bacterial concentration

The concentration and its range of culturable airborne bacteria in the four sampling sites in the university campus were showed in Figure 1. Considering all sampling area, the mean and geometric mean concentration of culturable airborne bacteria were 1224 and 1070 CFU/m^3 respectively in the university campus, Southeast China. Significant highest bacterial concentrations were found in LA, followed by DA and TA, while lowest fungal concentration was detected in OA (**P < 0.01). The mean bacterial concentration was about 1572 in LA, 1457 CFU/m^3 in DA, 1215 CFU/m^3 in TA, and 651 CFU/m^3 in OA.

Seasonal variation pattern of culturable airborne bacterial concentration

Significant differences in total bacterial concentrations among seasons existed in LA, DA and TA, where the mean concentrations were higher in summer (months

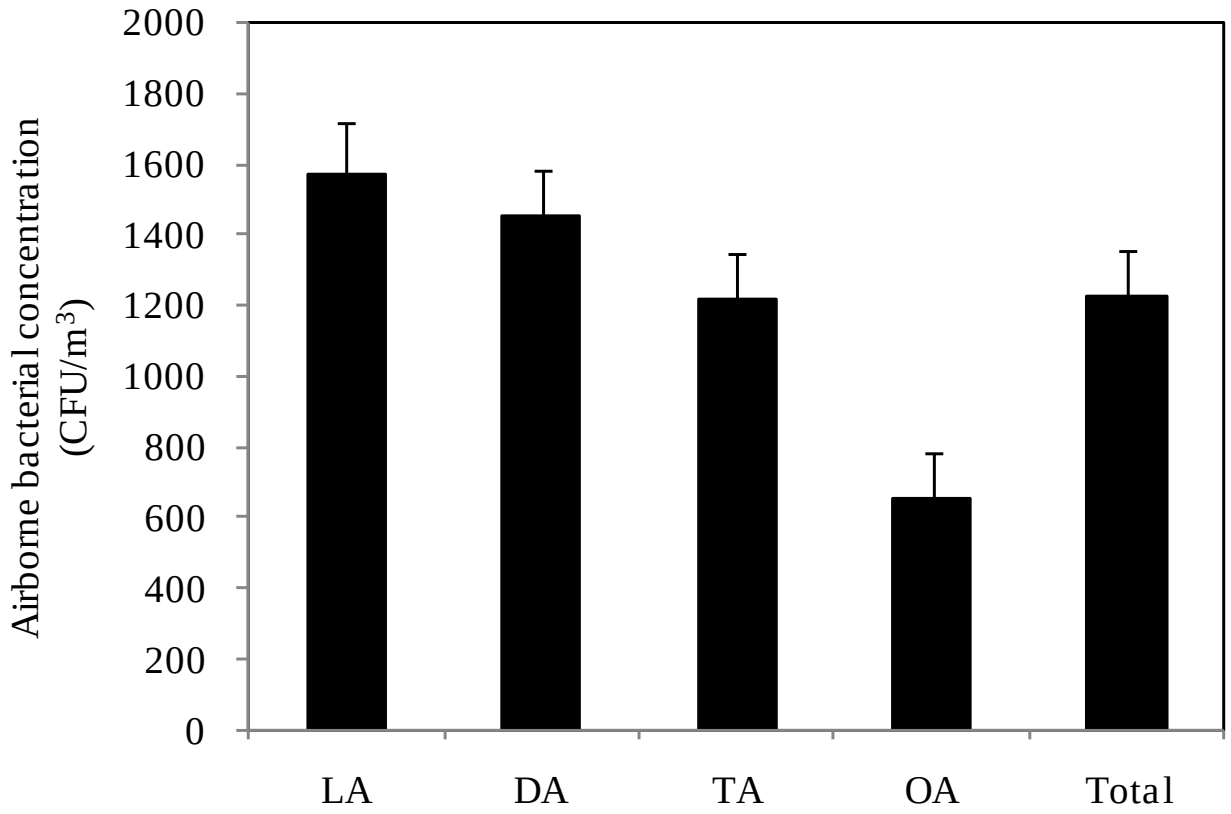

Figure 1. Variation pattern of airborne bacterial concentration in four sampling sites in the university campus.

from June to August) and autumn (months from Sep to Nov), and lower in spring (months from Mar to May) and winter (months from December to February) (**P < 0.01), while no significant variation of bacterial concentrations was observed in different seasons in OA (P > 0.05) (Figure 2).

Particle size distributions of culturable airborne bacteria in the university campus

Spatial variation pattern of particle size distribution

Particle size distribution of culturable airborne bacteria in the four sampling sites in the university campus was demonstrated in Figure 3. Basically, same bacterial size distribution pattern was observed in LA, DA, TA and OA in the university campus, and no significant difference of the size distribution pattern was found among these sampling areas (P > 0.05). The proportion of culturable airborne bacteria increased gradually from stage 1 (> 8.2 μm) to stage 5 (1.0 to 2.0 μm), and decreased drastically at stage 6 (< 1.0 μm). Most culturable airborne bacteria were distributed at stage 3 (3.0 to 6.0 μm), stage 4 (2.0 to 3.5 μm) and stage 5 in the sampler, totally contrubuting to 81.94, 84.20, 86.50, and 76.93% in the LA, DA, TA and OA, respectively, while just a few airborne bacteria were found at stage 1, stage 2 (5.0 to 10.4 μm) and stage 6 in the sampler. The highest proportions of culturable airborne bacteria were detected at stage 5 and the lowest at stage 6. The proportions were 44.73% (LA), 48.02% (DA), 46.23% (TA) and 39.22% (OA) at stage 5, and 3.35% (LA), 3.20% (DA), 3.06% (TA) and 4.89% (OA) at stage 6.

Seasonal variation pattern of particle size distribution

As same to particle size distribution of culturable airborne bacteria in the sampling areas, no significant difference of bacteria size distribution pattern was found among seasons in a year (P > 0.05) (Figure 4). The proportion of culturable airborne bacteria increased gradually from stage 1 (> 8.2 μm) to stage 5 (1.0 to 2.0 μm), and decreased drastically at stage 6 (< 1.0 μm). Most culturable airborne bacteria were distributed at stage 3 (3.0 to 6.0 μm), stage 4 (2.0 to 3.5 μm) and stage 5 in the sampler, totally contrubuting to 84.24, 81.92, 83.36, and 80.05% in spring, summer, autumn and winter, respectively. The highest proportions of culturable airborne bacteria were detected at stage 5 and the lowest at stage 6. The proportions were 45.9, 47.01, 46.78 and 38.43% (LA), (DA), (TA) (OA) respectively at stage 5, and 3.31% (LA), 3.29% (DA), 3.16% (TA) and 4.73%

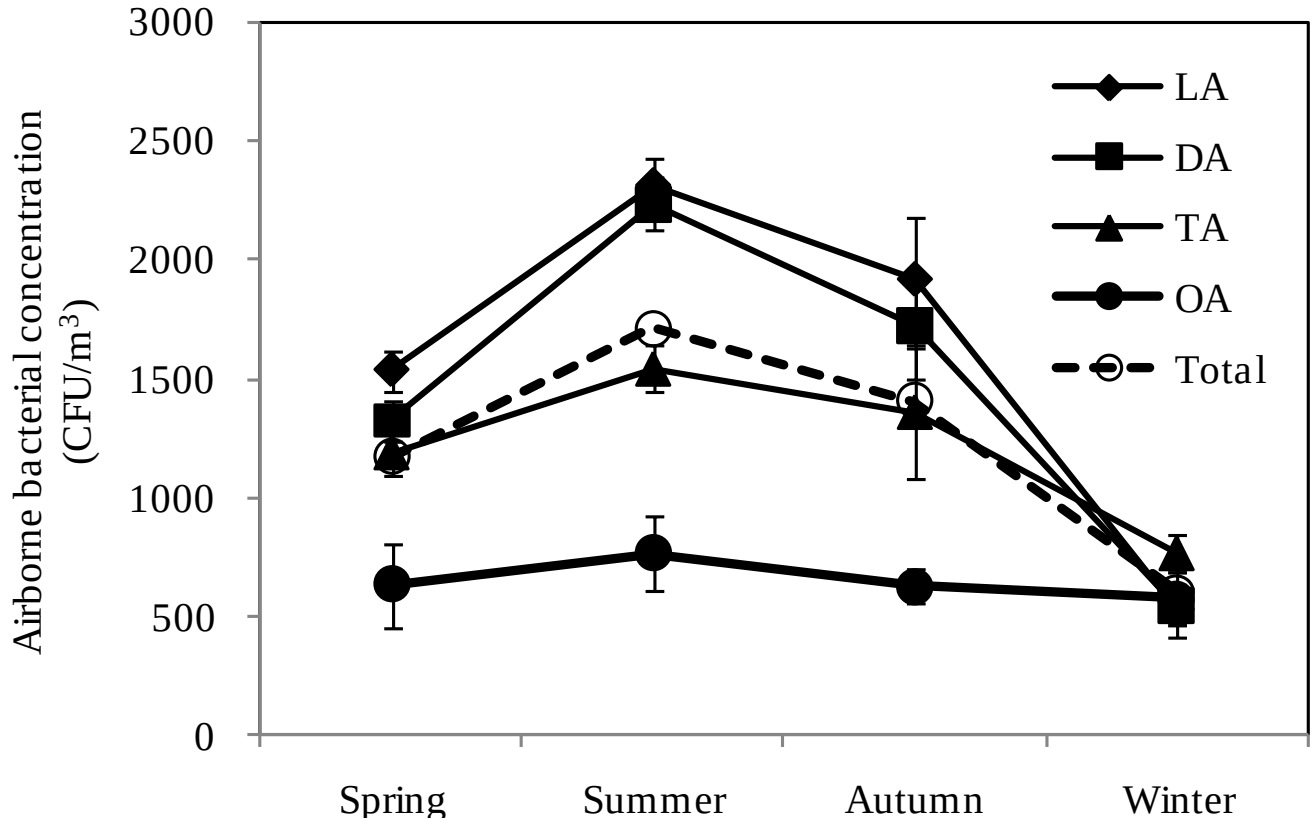

Figure 2. Seasonal variation pattern of airborne bacterial concentration in four sampling sites in the university campus.

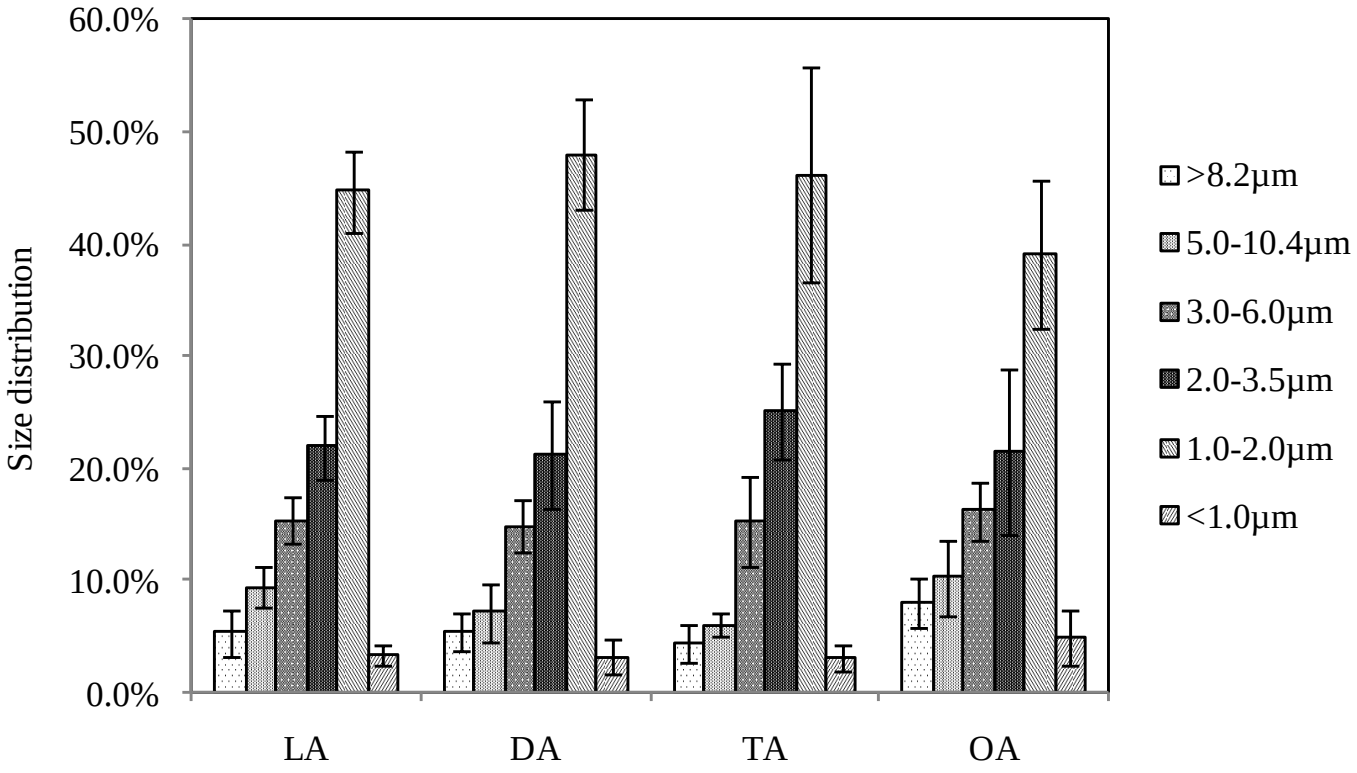

Figure 3. Size distribution of airborne bacteria in four sampling sites in the university campus.

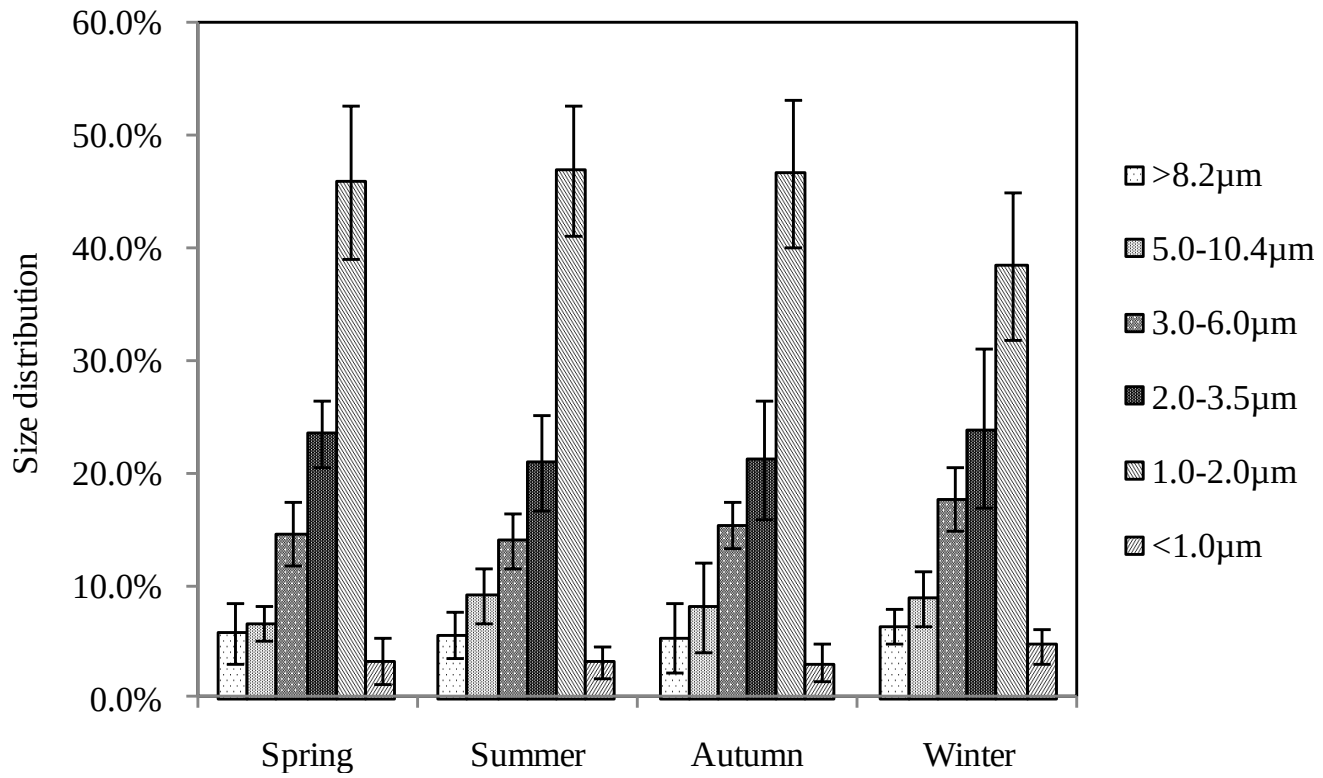

Figure 4. Size distribution of airborne bacteria in different seasons in a year in the university campus.

(OA) at stage 6.

DISCUSSION

The community composition, concentration variation, and size distribution of airborne culturable bacteria in LA, DA, TA, and OA in the university campus, southeast of China, was performed seasonally in a sampling year. Among a total of 533 bacterial colonies isolated from the samples, the number and concentration of airborne Gram-positive bacteria, accounting for about 84 to 90%, were significantly higher than that of airborne Gram-negative bacteria in indoor environments in university campus. It was consisted with our former results conducted in outdoor environments in Beijing, China (Fang et al., 2007), and also was in agreement with other reports (Shaffer and Lighthart, 1997; Zhu et al., 2003). Studies showed that more Gram-negative bacteria were found previously in the soil (Xie et al., 2004), which was one of the main sources of outdoor airborne bacteria, and anyway most indoor airborne bacteria comes from outdoor environments. The explanation of this conflict phenomenon was that Gram-positive bacteria in the air had greater resistance and survival ability than Gram-negative bacteria under strong sunlight (Xie et al., 1988), since air was not the ideal environment for microbial growth and reproduction due to shortness of the nutrient substrate in the air.

The most common bacteria groups in LA, DA, TA, and OA in the university campus were *Micrococcus*, *Bacillus*, *Staphylococcus*, *Corynebacterium*, and *Pseudomonas* according to priority, some of which had been reported as the most prevalent airborne bacteria in indoor environments in other studies, such as elementary school (Liu et al., 2000), crowded and underground public concourse (Seino et al., 2005), and university hospital (Sarica et al., 2002), child day care center (Aydogdu et al., 2010), feedstuff-manufacturing factories (Kim et al., 2009). Additionally, the most common bacteria in indoor air in the campus were consistent with our former findings carried out in indoor environments in Beijing, while some differences were also observed in the order of most common bacteria (Fang et al., 2007). In this study, *Bacillus* was the second most common bacteria in the indoor air, but in outdoor environments in Beijing, it was *Staphylococcus* which revealed as the second prevalent bacteria (Fang et al., 2007). This might be caused by the great differences of environmental conditions (such as human activities, culture substrate) and meteorological factors (such as precipitation, wind, solar radiation etc) between indoor and outdoor environments. *Micrococcus* and *Bacillus* dominated mostly in the indoor air in the campus since these airborne bacteria originated from outdoor environments, and the pigment of carotene in the genera of *Micrococcus* could resist the disinfection of UV

radiation, and the spores of *Bacillus* could suffer from arid environment and then germinate, which could result in the number increasing of *Micrococcus* and *Bacillus* outdoors and then indoors.

The present study demonstrated that the concentration of culturable bacteria in the university campus in Hangzhou, Southeast of China was with statistically significant differences in the selected four sampling sites. It was shown that bacterial concentration in LA was highest (1572 CFU/m^3), followed by DA (1457 CFU/m^3) and TA (1215 CFU/m^3), while the concentration in OA was lowest (651 CFU/m^3). Two sampling sites of LA and TA were with almost the same square area (20 m^2 Vs. 15 m^2) and both on the 2nd floor of the building, however, compared to OA, LA with six male graduate students seems more crowed ,with more personal activities and worse ventilation, and the room was not so clean because there are mess of cloths, socks, and other daily supplies, suggesting that human activities, cleanness, and ventilation were the major factors that influenced the concentration of indoor airborne bacteria. The same reasons were also for the larger space of DA and TA, for DA with many people and much leftover at the front of the door at the mealtime, and TA with many students for their classes frequently. As to its seasonal variation pattern, bacteria concentrations were higher in spring and summer, and lower in autumn and winter (**$P < 0.01$) in LA, DA, and TA, whereas there were no significant differences in bacterial concentrations in OA where the air-conditioner could keep the temperature relatively more stable with the change of seasons, indicating that air temperature was another important factor for airborne bacteria.

The size distribution of bacteria-associated aerosol particles was also assessed in this study. Our results showed that the proportion of culturable airborne bacteria increased gradually from stage 1 (> 8.2 μm) to stage 5 (1.0 to 2.0 μm), and decreased dramatically at stage 6 (< 1.0 μm). Previous studies on the size distribution of airborne bacteria were often carried out by using the six-stage cascade impactor. Lundholm (1982) reported that highest collection rates appeared at 1st, 2nd, 5th and 6th stage, while Macher et al. (1991) demonstrated highest collection rates at 1st and 5th stage. There were also several reports of size contribution of airborne bacteria in China both indoor and outdoor environments. For example, our previous study in outdoor environments in Beijing city revealed that the highest collection rates at 4th stage, and Wang et al. (2010) indicated that the highest collection rates in the Mogao Grottoes, Dunhuang, China was also at 4th stage. These results suggested that the size distribution of airborne bacteria differed greatly place to place. It has been hypothesized that bacteria attached to larger particles are more likely to retain culturability, perhaps because the particle protects them from environmental stresses (Lighthart, 2000).

However, the particle (especially the dust in air) size distribution rates were influenced by the specific environment of the sampling sites, such as the nature of soil, local climate, geography character, the coverage of plants and so on. Previous investigations indicated that the bacteria-associated aerosol particles median diameter at continental sites is about 4 μm, while at coastal sites it is about 2 μm (Shaffer and Lighthart, 1997; Tong and Lighthart, 2000; Wang et al., 2007). Therefore, it was reasonable that the highest collection rates in Hangzhou (coastal site in Southeast of China) was at 5th stage, whereas in Lanzhou (continental site in Northwest of China) and Beijing (continental site in North of China) were at 4th stage.

The problem of study only on culturable airborne bacteria has been taken consideration seriously, since the vast majority of environmental bacteria are nonculturable even when viable (Wainwright et al., 2004). The fraction of airborne bacteria that are detected by culture methods is typically less than 10%, with an observed range of 0.01 to 75%, and average values estimated at about 1% (Heidelberg et al., 1997; Lighthart 1998). Direct counting by epifluorescent microscopy (Kepner and Pratt, 1994; Harrison et al., 2005) and the quantitative polymerase chain reaction (Q-PCR) has been emerged in the area of airborne bacteria study (Hospodsky et al., 2010; Oppliger et al., 2008). Both methods are also of obvious disadvantages. For example, it is a tedious and time consuming process to directly count the bacteria by epifluorescent microscopy, because no fluorescent dye is specific to bacteria, and the bacterial cells must be counted by a human investigator taking into account the size and morphology of the stained particles. The method of Q-PCR is hard to differentiate the viable and dead bacteria efficiently and accurately. Here, we have measured the concentrations of airborne bacteria by collecting particulate matter via impaction on a culture medium and subsequently counting the colonies formed according to the State Standard of China (GB/T 18204.1 to 2000). However, we did agree that culture studies followed up with Q-PCR technique to obtain the quantitative results would be better. For airborne bacteria identification, we used culture methods, followed by sequencing and BLAST the 16S rRNA gene to determine the bacteria species. This molecular method of bacteria typing appeared to be excellent, for it could help to finish the identification work efficiently, with a high-throughput process referred to a standard operating procedure.

SUMMARY

In this study, an assessment of culturable airborne bacteria in indoor environments in the university campus was conducted seasonally and systemically in Hangzhou, southeast of China, we can conclude from the first hand data that (i) the major contributors to the indoor

environments in the university campus in Hangzhou, southeast of China, were *Micrococcus*, *Bacillus*, *Staphylococcus*, *Corynebacterium*, and *Pseudomonas* according to priority, and the genus of highest concentration was *Micrococcus*, (ii) lower airborne bacterial concentration in OA was detected than in LA, DA, and TA, where the bacterial concentration was higher in summer and autumn, but lower in spring and winter, and no seasonal variation pattern of bacterial concentration was observed in OA, (iii) the percentage of the culturable bacteria was gradually increased from stage 1 to 5, and then was dramatically decreased at stage 6 in the sampler, and most airborne bacteria was collected in stage 3, 4 and 5.

ACKNOWLEDGEMENTS

This study was financially supported by the Project of National Natural Science Foundation of China (No. 41005085), and the Project of Natural Science Foundation of Zhejiang Province (No. Y5100365), and the Open Fund Project of Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, and the Starting Research and Fund Project of Ministry of Education of returned overseas students, Authors also thank the teachers who provided their valuable suggestions and the graduate students who performed the microbial investigation in the campus air at Zhejiang Gongshang Univeristy, Hanghzhou, Zhejiang Province.

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