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Comprehensive characterization of indoor airborne bacterial profile

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Abstract

This is the first detailed characterization of the airborne bacterial profiles in indoor environments. Two restaurants were selected for this study. Fifteen genera of bacteria were isolated from each restaurant and identified by three different bacterial identification systems including MIDI, Biolog and Riboprinter®. The dominant bacteria of both restaurants were Gram-positive bacteria in which *Micrococcus* and *Bacillus* species were the most abundant. Most bacteria identified were representative species of skin and respiratory tract of human, and soil. Although the bacterial levels in these two restaurants were below the limit of the Hong Kong Indoor Air Quality Objective (HKIAQO) Level 1 standard (i.e., < 500 cfu/m^3), the majority of these bacteria were opportunistic pathogens. These results suggested that the identity of airborne bacteria should also be included in the IAQ to ensure there is a safety guideline for the public.

Key words: airborne bacterial profile; bacterial identification; indoor air quality
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Introduction

Indoor air quality (IAQ) has become a matter of growing concern over the past two decades (Etkin, 1994). In the past, most people just paid attention to the significant health effects caused by the outdoor air pollution; however, the exposure to the indoor pollutants by human has been increasing. According to the United States Environmental Protection Agency (USEPA), the levels of pollutants in the indoor environment may be 2–5 times, and occasionally more than 100 times, higher than the outdoor levels (USEPA, 1995). In addition, most people spend about 90% of their time indoors daily (USEPA, 1995). These findings have raised public awareness to the indoor air quality, and most people are trying to improve their IAQ to preserve their living standards and a better indoor environment.

There are three major types of indoor air contaminants; i.e., chemical, physical and biological (HKEPD, 1997, 1999; Lam, 2001). In the past, many studies only focused on the chemical pollutants in the indoor environments (Lam, 2001). Recently, biological pollutants including bacteria, fungi and viruses play an important role on defining the IAQ (Verdenelli *et al*., 2003) in which fungi are usually considered to be the major sources of microbial contamination in the indoor environments nowadays, but bacteria are usually ignored (Chao *et al*., 2002). There are only few detailed studies on indoor airborne microbes (Jaffal *et al*., 1997; Andersson *et al*., 1999; Camuffo *et al*., 1999; Pastuszka *et al*., 2000; Obbard and Fang, 2003). Moreover, many studies focused on the concentration of bacteria in the indoor environments and they just made use of those data as the indoor air quality indicator (HKEPD, 1997). Besides, global indoor air quality objectives including the Hong Kong Indoor Air Quality for IAQ management established in 1999 (HKEPD, 1999) also only focused on the concentration of bacteria in indoor air sample but not on the bacterial profile. Actually, it is important to know the types of bacteria presented in the indoor environments since airborne bacteria can cause disease transmission and allergic reaction to human (Cowan, 1974). Therefore, poor IAQ caused by the presence of some airborne bacteria is of increasing concern with respect to occupants' health (Obbard and Fang, 2003).

Nowadays, food premises are indispensable in our daily lives and people have to spend a significant portion of their time in the restaurants daily. Therefore, a good IAQ in restaurants does not only safeguard customers' health, but also provides a comfortable environment for the enjoyment of food. Therefore, two restaurants were chosen as models in this study to isolate and identify the bacteria in indoor air samples and to characterize the bacterial profiles.

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1 Materials and methods

1.1 Site selection

Two restaurants were selected to carry out the study. Restaurants are chosen because restaurants are public area in which different background of people (e.g., different age, sex, job nature, etc.) will be presented. Both restaurants are fast food restaurants and located on the hillside at The Chinese University of Hong Kong, Hong Kong SAR, China. Because the restaurants were air-conditioned during the working hours, the air temperature and humidity remained quite constant ((22 ± 2)°C; (60 ± 10)%). The canteens can accommodate 500 seats. There were usually around 20–30 people in the canteen after lunch time during sampling. No smoking activities were observed during the air sampling period.

1.2 Air sampling plan

A one-year study was conducted from March 2002 to January 2003 to collect 4 seasonal (March 2002, August 2002, November 2002, and January 2003) air samples. In each sampling, 1-h air sampling after lunch hours (around 2 p.m.) was carried out. All air samplers were put near to the center of the restaurant and at 1.5 m above the ground which are near the respiratory level of human. A SKC BioSampler® (SKC, Inc., USA) was used in this study (Lin *et al*., 1999). Twenty milliliter samples of sterilized ViaTrap® mineral oil (SKC, Inc., USA) were used for sampling and the flow rate of air was 12.5 L/min. After sampling, the SKC BioSamplers were sealed with aluminum foil and sent back to the laboratory for further investigation. Sample loss was not found by using the SKC BioSampler® (Lin *et al*., 1999).

1.3 Recovery of the air-borne bacteria

A rich medium, Difco™ Trytic Soy Agar (TSA) (Becton, Dickinson and Company, Sparks, USA) was used as the cultural medium (Parat *et al*., 1997; Pastuszka *et al*., 2000). The airborne bacteria were recovered by filtering the mineral oil through Millipore 0.45 μm sterile disposable filters (Millipore Corporation, Bedford, USA) and then parallel cultured on the TSA at 25 and 30°C (ACGIH, 1999; AIHA, 1996) and 37°C (for culturing human related bacteria), respectively, (20 mL mineral oil was averaged for 3 different temperatures) for 120 h. After 5 d of incubation, the number of colonies formed and their morphologies were recorded. Colonies with different morphologies (i.e., color, shape and texture, etc.) were picked and streaked on individual TSA plates. Three times of sub-culture were performed to obtain a pure culture and stabilize the characteristics of different bacterial strains.

1.4 Identification of bacterial strains

The enriched and purified bacterial strains were first subjected to microscopic examination and biochemical tests including Gram staining and oxidase test. Then three microbial identification methods were employed. If all the three methods identified a bacterium with the same genus and species name, the confirmed genus and species were used for the bacterium. If only two methods obtained the same genus and species name for a bacterium, only the genus name will be used. In this study, all bacteria were identified with the same genus and species name at least by two of the methods used.

1.4.1 MIDI identification

MIDI (MIDI Sherlock® Microbial Identification System, Newark, New Jersey, USA) identifies bacteria by their whole cell fatty acid profile. Methyl esters of fatty acids (FAMEs) were prepared and subjected to Hewlett-Packard 6890 gas chromatography analysis and compared with their HP A.06.X ChemStation software which has an extensive databases over 2000 species, including aerobic bacteria, anaerobic bacteria and yeasts.

1.4.2 Biolog MicroLog™ System

Biolog identifies bacteria by their substrate utilization pattern using the Biolog MicroStation reader (Biolog, Inc., Hayward, CA, USA) that was installed with a MicroLog™ software which consists of a database containing substrate utilization patterns of 1400 species of aerobic bacteria, anaerobic bacteria, and yeast.

1.4.3 Qualicon RiboPrinter® Microbial Characterization System

RiboPrinter identifies bacteria by analysis their 16S rRNA gene restriction digestion pattern. A 16–24 h culture was prepared by streaking the bacterial strain on TSA. Then, the colony was picked and suspended into the buffer solution and then subjected to the Qualicon™ heat treatment station (Qualicon, Inc., Wilmington, USA) which inactivates bacterial samples and renders them harmless. Next, 5 μL of lysing agent was added to each sample to lyse the cell. Finally, the heat treated samples was subjected to the characterization unit in which the sample would be undergone DNA restriction digestion, electrophoretic separation and membrane transfer. The 16S rRNA restriction digestion pattern generated was compared to the RiboPrinter® Microbial Characterization System (Qualicon, Inc., Wilmington, USA) analysis software which consists of a database containing Ribopattern for 120 genera and 1100 species.

2 Results

The bacterial profile and abundance of the samples are listed in Tables 1 and 2 for Restaurants 1 and 2, respectively. The bacterial strains were shown in an order according to their occurrences in the four seasonal samples.

There were totally 15 genera, 19 identified and 7 unidentified species of bacteria identified in the Restaurant 1 in which 8 genera were Gram-positive and 7 genera were Gram-negative. The frequently occurring genera presented in the Restaurant 1 including *Micrococcus*, *Bacillus* and *Pseudomonas* spp. *Micrococcus* species and *Bacillus* species appeared in all the samples.

For Restaurant 2, 15 genera, 16 identified and 7 uniden-

Table 1 Bacterial profile and their abundance of four seasonal samples in Restaurant 1

Genus	Occurrence (%)	Species	Number of bacteria (cfu/m^3 air sample)			
			Spring	Summer	Autumn	Winter
1. *Micrococcus* (+)	100	*luteus*	22	82	49	20
		lylae		5	13	1
2. *Bacillus* (+)	100	*pumilus*	1	1		3
		cereus		2		
		megaterium	2			2
		sp.			1	
3. *Pseudomonas* (–)	75	*stutzeri*		12		
		sp.	1		10	
4. Moraxella (–)	75	*osloensis*	2		4	3
		catarrhalis	4			
5. *Brevundimonas* (–)	75	*diminuta*		7	4	2
		vesicularis		2		1
6. Microbacterium (+)	75	*Imperiale*	4	13		
		sp.			5	
7. Kocuria (+)	75	*rosea*	1	2		
		kristinae				1
8. *Staphylococcus* (+)	50	*haemolyticus*			5	
		cohnii	9			
		epidermidis	4			
9. *Acinetobacter* (–)	50	*lwoffii*		7	2	
10. *Sphingomonas* (–)	25	sp.				1
11. *Brevibacterium* (+)	25	*casei*		4		
12. *Deinococcus* (+)	25	sp.				1
13. *Kytococcus* (+)	25	*sedentarius*	4			
14. *Rhodobacter* (–)	25	sp.				2
15. *Paracoccus* (–)	25	sp.			2	
Total number of species identified			11	11	10	11
Total number of bacteria identified (cfu/m^3 air)			54	137	95	37

The percentage occurrence of the bacterial genus in the four seasonal samples.
+ and – represent Gram-positive and Gram-negative, respectively.

Table 2 Bacterial profile and their abundance of four seasonal samples in Restaurant 2

Genus	Occurrence (%)	Species	Number of bacteria (cfu/m^3 air sample)			
			Spring	Summer	Autumn	Winter
1. *Micrococcus* (+)	100	*luteus*	15	76	27	19
		lylae	1			4
2. *Bacillus* (+)	100	*pumilus*		5	1	1
		thuringiensis		1		
		sp.	1			
3. *Brevundimonas* (–)	100	*diminuta*	1	5	15	
		vesicularis				5
4. *Staphylococcus* (+)	75	*hominis*	3	9		
		epidermidis	1			
		warneri	1			
		sp.			4	
5. *Pseudomonas* (–)	50	*stutzeri*		1		
		sp.				3
6. *Arthrobacter* (–)	50	sp.	1		1	
7. *Acinetobacter* (–)	50	*lwoffii*			7	9
9. *Curtobacterium* (+)	50	sp.	1			1
8. *Stenotrophomonas* (–)	25	*maltophilia*			41	
10. *Moraxella* (–)	25	*osloensis*		4		
11. *Kocuria* (+)	25	*kristinae*			9	
12. *Microbacterium* (+)	25	*lmperiale*				1
13. Chryseobacterium (–)	25	sp.		1		
14. *Sphingomonas* (–)	25	*capsulata*				3
15. *Rhodococcus* (+)	25	sp.		1		
Total number of species identified			9	9	8	9
Total number of bacteria identified (cfu/m^3 air)			25	103	105	46

The percentage occurrence of the bacterial genus in the four seasonal samples.
+ and – represent Gram-positive and Gram-negative, respectively.

tified species of bacteria identified in Restaurant 2 in which 7 genera were Gram-positive and 8 genera were Gram-negative (Table 2). Similar to Restaurant 1, *Micrococcus* species and *Bacillus* species appeared in all the samples.

The bacterial concentrations were all below 500 cfu/m^3 of air, i.e., below the limit of the Hong Kong Indoor Air Quality Objective (HKIAQO) Level 1 standard (i.e., < 500 cfu/m^3).

3 Discussion

It is the first study, in which a comprehensive identification of the airborne bacteria in an indoor environment was performed. Therefore, the results of this study can serve as a model for air sampling and detail bacterial profile analysis in other indoor environments. There are several unique characteristics of both bacterial profiles of Restaurants 1 and 2 (Tables 1 and 2). Gram-positive bacteria, especially *Micrococcus* and *Bacillus* species, were dominant in the sampling sites. In the air-conditioned indoor environment, the major problem the bacteria encountered will be the limitation of water content (Etkin, 1994). Since Gram-positive bacteria have thick peptidoglycan layers on their cell wall, it can protect them against desiccation and they can survive the dry condition (Etkin, 1994). Moreover, *Micrococcus* species contain carotenoid pigments which can protect them from damages by the ultraviolet (UV) radiation from sunlight (Etkin, 1994); and the *Bacillus* species can form endospores, allowing them to tolerate harsh (e.g., dry) environment and easily disperse in air. In addition, the bacteria isolated were usually of human and soil origins. The bacteria that come from human are mainly from the skin and respiratory tract, and were released by occupants into the sites through shedding off from skin, sneezing or talking; whereas the soil bacteria were usually dispersed in the air by dust. Therefore, the bacteria isolated from the sampling sites were contributed by human and the outdoor environment. Finally, the majority of the bacteria was either opportunistic pathogens or non-pathogenic, and they would cause diseases only in sensitized or grossly immuno-compromised individuals, with the exception of *Moraxella catarrhalis* which is usually considered pathogenic (Cowan, 1974; Krieg *et al*., 1984; Sneath *et al*., 1986; Williams *et al*., 1989; Holt *et al*., 1994; Segers *et al*., 1994; Bascomb and Manafi, 1998; Govan *et al*., 1999; Chung *et al*., 2000; Tan and Grewal, 2001; Becker *et al*., 2002; Basaglia *et al*., 2002; Verduin *et al*., 2002; Laffineur *et al*., 2003; Riffel *et al*., 2003; Tekerekoglu *et al*., 2003; Wolf *et al*., 2003).

From Tables 1 and 2, the results showed that similar bacterial species appeared with different sampling seasons and different sampling sites. It can be explained by that the types (mainly students) and activities (eating) of the occupants were similar in both restaurants which may contribute similar bacteria to the environments. Therefore, the air sampling season would not affect the bacterial diversity in the environment in a great extent. However, the results showed that the bacterial concentration fluctuated with different sampling seasons. Obviously, the bacterial concentration was affected by the seasonal differences as the four samples represent four seasons (i.e., spring, summer, autumn and winter). Tables 1 and 2 showed that the bacterial concentration greatly increased during summer and autumn, this indicates the air quality in hotter seasons will become poorer and this may enhance the sick building syndromes of the customers. Therefore, the selection of sampling season is an important factor affecting the air quality in the restaurant environment.

As mentioned in the introduction, although the worldwide indoor air quality guidelines concerned only about the bacterial concentration and cannot safeguard wellbeing as the bacterial type presented in the indoor environment, it also plays an important role on the determination of indoor air quality. By using the results of this study as a model, the bacterial concentrations were all below the HKIAQO Level 1 standard (i.e., < 500 cfu/m^3). According to the HKIAQO, Level 1 standard represents a very good indoor air quality which high-class and comfortable buildings should have. However, some bacterial species presented in these restaurant environments were pathogens and some were opportunistic pathogens such as *Moraxella catarrhalis* which is associated with upper respiratory tract infections in healthy children and elderly people; lower respiratory tract infections in adults with chronic obstructive pulmonary disease; pneumonia, endocarditis, septicemia and meningitis in immunocompromized hosts. Therefore, besides the bacterial concentration, the type of bacteria presented in the indoor environments should also be provided in the indoor air quality guidelines.

4 Conclusions

After the comprehensive identification of the airborne bacteria in the two restaurants, the results indicated that Gram-positive bacteria were dominant in the indoor air of the restaurants in which *Micrococcus* and *Bacillus* species were the most frequently found bacterial species. The sources of the isolated bacteria were usually from human and soil, and the major groups of the bacteria were opportunistic pathogens. Although the bacterial levels were below the limit of the HKIAQO Level 1 standard (i.e., < 500 cfu/m^3), but there is certain hazard for people to visit these restaurants since some pathogens and/or opportunistic pathogens were identified in the air samples. The results of this study strongly suggested that the bacterial identification should also be included in the preparation of the revised IAQ.

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