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ORIGINAL PAPER

Viable airborne microbial counts from air-cooling units with and without complaints of urine and body odors

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Abstract Viable airborne microbial counts are commonly used in indoor air quality (IAQ) assessment, but studies linking the microbial counts to a specific type of indoor microbial contamination are limited. We hypothesize that the airborne microbial counts can differentiate air-cooling units with and without complaints of urine and body odors. The keratinolytic property of some isolated bacteria prompts to the hypothesis that keratinase is present in the units to break down keratins, structural proteins that form human skin scales, as sources of amino acids and ammonium to produce the odors. Seven bacterial species and four fungal species were identified in the units and room air. Airborne *Staphylococcus haemolyticus* and *Methylobacterium organophilum* counts contributed the most to the microbial dissimilarities of units with and without odor complaints. Keratinolytic bacteria and a methylotrophic bacterium were abundant in the units. All the units contained ammonium, and keratinase activity was higher in the units with odor complaints. Extracellular keratinase activity was more effective at 20 °C than at 30 or 4 °C. Keratinolytic bacteria produced high levels of ammonium in the culture with skin cells. Viable airborne microbial counts can help IAQ inspectors to identify potential odor-causing air-cooling units. Keratins may be broken down in the units and associated with the odor complaints.

Keywords Indoor air quality · Microbial odor emission · Air-conditioning systems · Bioaerosols

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1 Introduction

Viable airborne microbial counts are commonly used in indoor air quality (IAQ) assessment, but studies linking the microbial counts to a specific type of indoor microbial contamination are limited (HKEPD 2003). Air-conditioning systems (ACSs) are necessary to provide comfortable indoor environments in many parts of the world. The maintenance of a healthy system is an important building health and sustainability issue. The emission of odors from ACSs can become a serious disturbance to building's occupants by affecting their health and well-being (e.g., sick-building syndrome) and by reducing their productivity (Kosonen and Tan 2004; Barkat et al. 2012). Different origins (e.g., chemical or biological) and types of odor complaints have been reported in association with ACSs (Bluyssen et al. 2003; Beko et al. 2006). Bacterial and fungal contamination can cause odor problems. It is reasonable to hypothesize that bacterial

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and fungal contamination in ACSs can be detected using viable airborne microbial counts because bacteria and fungi can be dispersed into the air (Hugenholtz and Fuerst 1992).

Complaints of urine-like and body odors from air-cooling units have been received on our university campus. After building and engineering investigations, it is suspected that the odors may have a microbiological origin. Measuring the viable airborne microbial counts is the standard protocol used by the building and health inspectors to routinely monitor IAQ and investigate IAQ problems. However, no study has been conducted to link the microbial counts to odor complaints. The IAQ guidelines used in the university campus are adopted from the Hong Kong Environmental Protection Department (HKEPD) IAQ Objectives for Offices and Public Places (HKEPD 2003). According to the IAQ objectives, indoor air with total bacterial counts <500 CFU/m^3 is considered to be excellent and <1000 CFU/m^3 is good. These guidelines are inadequate to help inspectors to identify potential odor-causing units. Therefore, the aim of this study is to investigate and compare the microbial species sampled from air-cooling units with and without odor complaints and so to improve the current IAQ inspection. After studying the biological properties of our isolated bacteria, we also hypothesize that keratins, structural proteins, that form human skin scales can accumulate in the air-cooling units and play a role in the odor production.

2 Materials and methods

2.1 Site information

Table 1 shows the functions of the rooms that contained the examined air-cooling units. The air-cooling unit is composed of aluminum evaporator fins that cool the air. A drain pan is installed underneath to collect the condensed water, and a plastic tube connected to the drain pan discharges the water. Four units that had odor complaints at the time of our investigation and six units with no odor complaints or complaints only in the past were examined. All units for which odor was reported had been checked by the investigation team with ACS engineers and maintenance staff and were considered to be visibly clean and to have no other notable sources of smell in the system. The fresh air supply source, the air inlet filtering unit, and the duct system had all been checked.

2.2 Air and water sampling

Because the air-cooling unit is fixed in the system, pressurized tap water was used to spray the aluminum evaporator fins in the air-cooling unit for 4–5 min to dislodge microbes from the fins, which is the practice used by the maintenance staff to clean the unit, except that cleaning agents are usually used instead of water alone. Microorganisms were collected in both air and water samples. For air samples, 200 L of air was sampled using a handheld air sampler (Pbi air sampler SAS Super 100) with either trypticase soy agar for bacterial culture or potato dextrose agar for fungal culture before (i.e., to indicate background microbial levels) and during the process of spraying the unit (i.e., to indicate the microbial levels dislodged from the fins and released from the air outlet). Duplicate, instead of triplicate, samples were taken because of the size of the rooms. Water samples were collected from the drain pan; therefore, the microbes dislodged from the fins and other internal part of the unit and preexisted in the drain pan were sampled. Because the instruments and apparatus used to prepare the pressurized water cannot all be sterilized before use, the bacterial, fungal, and ammonium levels in the pressurized tap water were measured as blanks to account for the background levels. After the spraying process, all of the water in the drain pan was collected, and the total volume was recorded to calculate the levels of bacteria, fungi, and ammonium eluted per air-cooling unit. An aliquot of the water sample was taken for bacterial and fungal plate counting using the corresponding agar media. The trypticase soy agar plates were incubated at 30 °C for 48 h, and the potato dextrose agar plates were incubated at 25 °C for 96 h before counting. These microbial culture methods are currently used in the IAQ inspection. Bacterial and fungal colonies were separated morphologically and counted. Individual bacterial colonies were selected and subcultured for sequencing using Applied Biosystems 3730*xl* DNA Analyzer with 16S Amplicon Forward and 16S Amplicon Reverse primers (Anesti et al. 2004). Fungal colonies were identified

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Table 1 Room function, bacterial and fungal counts in each unit and air sample, and ammonium levels in the units

Room function (last cleaned, months)	Microbes eluted from the unit, water samples (CFU/unit)		Microbes in air before/during spray (CFU/m^3)		NH_4^+–N from the unit (mg)
Odor reported currently					
Pantry (6)	*S. haemolyticus*: 706	*P. chryosgenum*: 1765	*S. haemolyticus*: 100/205	*P. chryosgenum*: <5/30	0.033
	M. organophilum: 1589	*M. ramosissimus*: 3531	*M. organophilum*: 10/75	*M. ramosissimus*: 10/40	
	M. luteus: 0	*A. fumigatus*: 3531	*M. luteus*: 5/<5	*A. fumigatus*: 10/20	
	B. thuringiensis: 706	*P. uncinulatum*: 8827	*B. thuringiensis*: 5/25	*P. uncinulatum*: <5/50	
	S. paucimobilis: 353		*S. paucimobilis*: 15/60		
	S. epidermidis: 0		*S. epidermidis*: <5/5		
	L. varians: 0		*L. varians*: 40/100		
Lift lobby 1 (12)	*S. haemolyticus*: 765	*P. chryosgenum*: 0	*S. haemolyticus*: 75/185	*P. chryosgenum*: 20/105	0.297
	M. organophilum: 1530	*M. ramosissimus*: 9563	*M. organophilum*: 45/100	*M. ramosissimus*: 35/60	
	M. luteus: 383	*A. fumigatus*: 0	*M. luteus*: 5/20	*A. fumigatus*: 40/50	
	B. thuringiensis: 191	*P. uncinulatum*: 3825	*B. thuringiensis*: 10/20	*P. uncinulatum*: 50/100	
	S. paucimobilis: 1148		*S. paucimobilis*: 10/75		
	S. epidermidis: 0		*S. epidermidis*: <5/<5		
	L. varians: 383		*L. varians*: 30/95		
Open-plan office 1 (2)	*S. haemolyticus*: 470	*P. chryosgenum*: 3918	*S. haemolyticus*: 105/245	*P. chryosgenum*: 20/235	0.148
	M. organophilum: 1959	*M. ramosissimus*: 9794	*M. organophilum*: 75/125	*M. ramosissimus*: 20/135	
	M. luteus: 588	*A. fumigatus*: 0	*M. luteus*: 5/20	*A. fumigatus*: 30/290	
	B. thuringiensis: 548	*P. uncinulatum*: 7836	*B. thuringiensis*: 10/20	*P. uncinulatum*: 5/20	
	S. paucimobilis: 979		*S. paucimobilis*: 5/20		
	S. epidermidis: 0		*S. epidermidis*: <5/<5		
	L. varians: 176		*L. varians*: 40/115		
Project room 1 (1.5)	*S. haemolyticus*: 1018	*P. chryosgenum*: 5504	*S. haemolyticus*: 200/330	*P. chryosgenum*: 50/40	0.055
	M. organophilum: 1651	*M. ramosissimus*: 8256	*M. organophilum*: 65/175	*M. ramosissimus*: 10/10	
	M. luteus: 688	*A. fumigatus*: 0	*M. luteus*: 25/20	*A. fumigatus*: 30/10	
	B. thuringiensis: 248	*P. uncinulatum*: 1376	*B. thuringiensis*: 35/30	*P. uncinulatum*: 5/5	
	S. paucimobilis: 688		*S. paucimobilis*: 45/95		
	S. epidermidis: 0		*S. epidermidis*: <5/<5		
	L. varians: 261		*L. varians*: 160/155		

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Table 1 continued

Room function (last cleaned, months)	Microbes eluted from the unit, water samples (CFU/unit)		Microbes in air before/during spray (CFU/m^3)		NH_4^+–N from the unit (mg)
No-odor reported currently					
Open-plan office 2 (6)	*S. haemolyticus*: 545 *M. organophilum*: 727 *M. luteus*: 363 *B. thuringiensis*: 182 *S. paucimobilis*: 908 *S. epidermidis*: 0 *L. varians*: 908	*P. chryosgenum*: 3634 *M. ramosissimus*: 0 *A. fumigatus*: 0 *P. uncinulatum*: 9084	*S. haemolyticus*: 55/135 *M. organophilum*: 5/45 *M. luteus*: 5/25 *B. thuringiensis*: 5/15 *S. paucimobilis*: 15/55 *S. epidermidis*: 5/20 *L. varians*: 45/115	*P. chryosgenum*: 10/50 *M. ramosissimus*: 10/90 *A. fumigatus*: 10/100 *P. uncinulatum*: 10/40	0.170
Activity room 1 (6)	*S. haemolyticus*: 720 *M. organophilum*: 1621 *M. luteus*: 0 *B. thuringiensis*: 720 *S. paucimobilis*: 360 *S. epidermidis*: 0 *L. varians*: 0	*P. chryosgenum*: 3602 *M. ramosissimus*: 1801 *A. fumigatus*: 1801 *P. uncinulatum*: 7205	*S. haemolyticus*: 65/165 *M. organophilum*: 15/65 *M. luteus*: 10/20 *B. thuringiensis*: 5/20 *S. paucimobilis*: 5/45 *S. epidermidis*: <5/<5 *L. varians*: 30/105	*P. chryosgenum*: 5/45 *M. ramosissimus*: 5/30 *A. fumigatus*: 5/50 *P. uncinulatum*: 5/55	0.018
Activity room 2 (6)	*S. haemolyticus*: 636 *M. organophilum*: 847 *M. luteus*: 424 *B. thuringiensis*: 212 *S. paucimobilis*: 1059 *S. epidermidis*: 0 *L. varians*: 1059	*P. chryosgenum*: 2119 *M. ramosissimus*: 2119 *A. fumigatus*: 2119 *P. uncinulatum*: 6358	*S. haemolyticus*: 45/130 *M. organophilum*: 5/75 *M. luteus*: 5/10 *B. thuringiensis*: 5/15 *S. paucimobilis*: 5/55 *S. epidermidis*: <5/<5 *L. varians*: 20/85	*P. chryosgenum*: 5/50 *M. ramosissimus*: <5/20 *A. fumigatus*: 5/60 *P. uncinulatum*: 5/60	0.155
Project room 2[a] The same odor reported project room above but different units (1.5)	*S. haemolyticus*: 372 *M. organophilum*: 876 *M. luteus*: 329 *B. thuringiensis*: 88 *S. paucimobilis*: 329 *S. epidermidis*: 0 *L. varians*: 99	*P. chryosgenum*: 2190 *M. ramosissimus*: 2190 *A. fumigatus*: 0 *P. uncinulatum*: 0	Same project room above, so no air sample taken when spraying this unit to avoid cross-sample contamination		0.034

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Table 1 continued

Room function (last cleaned, months)	Microbes eluted from the unit, water samples (CFU/unit)		Microbes in air before/during spray (CFU/m^3)		NH_4^+–N from the unit (mg)
Lift lobby 2 (12)	*S. haemolyticus*: 548	*P. chryosgenum*: 0	*S. haemolyticus*: 50/125	*P. chryosgenum*: 20/55	0.095
	M. organophilum: 1096	*M. ramosissimus*: 5480	*M. organophilum*: 5/60	*M. ramosissimus*: 10/20	
	M. luteus: 365	*A. fumigatus*: 0	*M. luteus*: 2/10	*A. fumigatus*: 30/85	
	B. thuringiensis: 183	*P. uncinulatum*: 9133	*B. thuringiensis*: 5/5	*P. uncinulatum*: 25/60	
	S. paucimobilis: 731		*S. paucimobilis*: 5/70		
	S. epidermidis: 0		*S. epidermidis*: <5/<5		
	L. varians: 548		*L. varians*: 35/85		
Vacant office[b] (24)	Mold covered the plates	Overloaded with mold	*S. haemolyticus*: 150/mold	*P. chryosgenum*: 60/150	0.122
			M. organophilum: 30/mold	*M. ramosissimus*: 60/150	
			M. luteus: 20/mold	*A. fumigatus*: 95/110	
			B. thuringiensis: 25/mold	*P. uncinulatum*: 50/145	
			S. paucimobilis: 55/mold		
			S. epidermidis: <5/mold		
			L. varians: 135/mold		

[a] Odor reported previously, located in the same room as the currently odor reported unit

[b] Very serious and continuous odor complaints even after several sections of cleaning; the room has been vacant with the air conditioner turned off for a year because of the odor

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morphologically by spore-forming structure and ITS86-ITS4 primers (Tananuvat et al. 2012). After BLAST alignment, the bacterial and fungal species were identified as those with a sequencing match above 98% and with only a single species shown. For the identification did not meet these criteria, species-specific primers were used to confirm the microbial identity. The ammonium levels in the water samples were measured using the indophenol blue method, and the final ammonium level eluted from each unit was calculated.

2.3 Methylotrophic and keratin degradation activity

Methanol (AR Grade, RCI Labscan) was added to mineral salt agar to achieve a concentration of 0.5% as the sole carbon source to identify methylotrophic bacteria (Uy et al. 2013). The bacteria were washed with phosphate-buffered saline solution to remove any residual nutrient, inoculated onto the agar plates, and incubated at 30 °C for 1 week. The presence of colonies indicated the methylotrophic activity of the bacteria. To test the keratinolytic activity, individual bacteria were grown to a stationary phase in trypticase soy broth in a 30 °C incubator shaker operating at 150 rpm. Afterward, the bacteria were washed three times with modified Spizizen's minimal medium [0.02% $MgSO_4$ ($7H_2O$), 0.6% KH_2PO_4, 1.4% K_2HPO_4, 0.1% sodium citrate ($2H_2O$), 0.5% glucose, 0.1 μg/mL biotin], and the final bacterial concentration suspended in Spizizen's minimal medium was adjusted to approximately 10^6 colony-forming units per mL (CFU/mL). About 0.2 g of human skin scales were added to 100 mL of the medium and incubated for 4 days in the same incubator shaker. The skin scales were donated by a healthy volunteer without any known skin problem. It is a regular practice of the volunteer to remove dead skin scales from feet using an electric foot file. The skin scales had been air-dried under germicidal UVC irradiation for 2 days to inactivate the indigenous skin flora. To rule out a false-positive result, controls with skin scales only were added to the medium and incubated in parallel with the other test cultures. During incubation, bacterial growth was determined by measuring the increase in culturable counts and the optical density (OD_{600}) of the bacterial culture. No bacterial growth was detected in the skin scales only medium. The ammonium level in the culture was measured with the indophenol blue method. After incubation, the bacterial culture was filtered through a 0.2-μm cellulose acetate membrane filter to extract the extracellular enzymes, e.g., keratinase. The protein levels in the filtrates were determined with the method developed by Bradford (Bressollier et al. 1999). This protein level was used to calculate the yield of keratinase among the total extracellular protein content (Bressollier et al. 1999). About 4 mg of keratin azure (Sigma, USA) was added into 2 mL of filtrates and incubated for 12 days at 4, 20, and 30 °C to determine the activity of keratinase at different temperatures. After incubation, the change in the color of the culture was measured at 595 nm; dyes are released when keratinase breaks down keratin azure (Bressollier et al. 1999). All experiments were conducted in triplicate. Keratinolytic activity of the water samples eluted from the air-cooling units was determined using the same method with keratin azure and incubated at 30 °C.

2.4 Data analysis

The abundance of each microbial species in both air and water samples in units with and without odor complaints was compared with the Wilcoxon rank sum test (SPSS Statistics v23). The abundance of microbial species in air samples before and during the spraying process was compared using Wilcoxon signed rank test (SPSS Statistics v23). To investigate potential odor-causing microbes and/or communities, we examined the differences in the composition of bacterial and fungal communities by nonmetric multidimensional scaling using Primer software (Clarke and Warwick 2001). Analysis of dissimilarity was used to test for significant differences of bacterial and fungal compositions between units with and without odor complaints (Clarke and Warwick 2001). For dissimilar compositions, we applied similarity percentage analysis to calculate the contribution of individual groups and/or species toward the differences in the communities. A similar analysis was applied to compare the community composition between the units and air samples and the air samples before and during the spraying process. The mean ammonium levels in units with and without odor complaints were compared with an independent-sample *t* test (SPSS Statistics v23). Spearman's rank correlation coefficient (SPSS Statistics v23) was used to correlate the counts of bacterial and fungal species in the unit and in the air.

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3 Results

3.1 Microbial identification and microbial communities in units with and without odor complaints

Seven bacterial species (*Staphylococcus haemolyticus*, *Methylobacterium organophilum*, *Micrococcus luteus*, *Bacillus thuringiensis*, *Sphingmonas paucimobilis*, *Staphylococcus epidermidis*, and *Lysinibacillus varians*) and four fungal species (*Penicillium chryosgenum*, *Mucor ramosissimus*, *Aspergillus fumigatus*, and *Pythium uncinulatum*) were identified in this study. Table 1 shows the diversity and abundance of the bacteria and fungi collected in the air samples and eluted from each unit after deducting individual bacterial and fungal count in the corresponding blank. The total bacterial and fungal contamination levels in the blanks were always lower than 10 CFU/100 mL and 300 CFU/100 mL, respectively. These microbial levels were much lower than that found in the eluents from the air-cooling units. In general, *S. haemolyticus* was frequently found in the blanks, and its level was on average three times lower than that in the eluents from no smell units. Low levels of *B. thuringiensis* and *L. varians* were sometimes found in some blanks, too. For fungi, all the fungal species could be found in the blanks but less frequent for *P. uncinulatum*, at very low levels compared to the fungi in the eluents. For the microbes eluted from the units (water samples), the abundance of all bacteria in the units with odor complaints was marginally higher than that of those without odor complaints ($W = 4$; $p = 0.052$). The community composition was not different between the units with and without odor complaints for both bacteria ($R = 0.030$; $p = 0.086$) and fungi ($R = -0.028$; $p = 0.520$). In the air samples taken before the spraying process (background levels), the difference in the level of *M. organophilum* between the units with and without odor complaints was marginally significant ($W = 2$; $p = 0.062$). The fungal composition was similar between the units with and without odor complaints ($R = 0.177$; $p = 0.171$), whereas the difference in the bacterial composition was marginally significant in the air samples ($R = 0.406$; $p = 0.057$). *M. organophilum* and *S. haemolyticus* contributed the highest percentages (*M. organophilum*, 33.3%; *S. haemolyticus*, 16.6%) to the dissimilarities in the bacterial composition in the background air samples between the units with and without odor complaints.

During the spraying process, the difference between the bacterial compositions from the units with and without odor complaints was significant ($R = 0.438$; $p = 0.029$). *S. haemolyticus* and *M. organophilum* contributed the most (*S. haemolyticus*, 26.5%; *M. organophilum*, 21.1%) to the dissimilarities in the bacterial composition between the units with and without odor complaints. The abundance of *S. haemolyticus* ($W = 16$; $p = 0.03$), *M. organophilum* ($W = 15.5$; $p = 0.04$), and total bacterial colonies ($W = 16$; $p = 0.03$) were significantly higher in units with odor complaints than in those without odor complaints, whereas the levels of *M. luteus*, *B. thuringiensis*, *S. paucimobilis*, *S. epidermidis*, and *L. varians* were similar. The difference between the fungal compositions from these units was not significant ($R = 0.138$; $p = 0.183$). The abundance of all fungal species and total fungal colonies were similar between units with and without odor complaints.

The abundance of *S. haemolyticus*, *M. organophilum*, *B. thuringiensis*, *S. paucimobilis*, *L. varians*, *P. chryosgenum*, *M. ramosissimus*, and *P. uncinulatum* increased significantly when the air-cooling units were sprayed (Table 2). These results support the notion that these microbes originated from the evaporator fins and the air outlet components. The microbial compositions differed between the air samples taken before and during the spraying process both for bacteria ($R = 0.655$; $p = 0.003$) and for fungi ($R = 0.238$; $p = 0.012$). *S. paucimobilis* and *M. organophilum* contributed the highest percentages (22.1 and 22.0%, respectively) to the dissimilarities in the bacterial composition, whereas *P. chryosgenum* and *P. uncinulatum* contributed the highest percentages (22.6 and 21.4%, respectively) to the dissimilarities in the fungal compositions. These results imply that these microbes may have lower emission rates per population than other species during the normal operation of the air-cooling unit.

3.2 Interspecies correlation and correlation with ammonium levels eluted from the units

Table 1 shows the ammonium level eluted from each unit. The ammonium levels between the units with (average = 0.13 mg) and without odor complaints (average = 0.09 mg) units were not statistically different. However, the ammonium levels were strongly correlated with the elution of *S. paucimobilis*

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Table 2 Average abundance of microbes in the air samples obtained before and during spray

Microbes	Before	During	*P*
S. haemolyticus	2.81	3.61	0.008*
M. organophilum	1.53	2.87	0.014*
M. luteus	0.88	1.30	0.076
B. thuringiensis	0.97	1.50	0.031*
S. paucimobilis	1.12	2.49	0.014*
S. epidermidis[a]	0.13	0.63	0.371
L. varians	2.22	3.09	0.021*
Total bacteria	39.38	96.75	0.014*
P. chryosgenum	1.39	2.68	0.013*
M. ramosissimus	1.28	2.30	0.014*
A. fumigatus	1.62	2.58	0.033*
P. uncinulatum	1.16	2.33	0.014*
Total Fungi	2.55	3.88	0.008*

* Statistically significant at $p < 0.05$

[a] Limited samples sizes

($r = 0.883$; $p = 0.002$) and *L. varians* ($r = 0.812$; $p = 0.008$) from the units. The ammonium levels were also correlated ($r = 0.714$; $p = 0.047$) with the total fungal counts in the air samples during the spraying process, but no correlation was observed with any microbe in the samples taken before the spraying process. Table 3 shows the significant interspecies correlation within each sample set. The counts of *S. haemolyticus*, *M. organophilum*, and *B. thuringiensis* showed strong correlations with each other in both air samples, whereas in the water samples, *M. organophilum* and *B. thuringiensis*, and *S. paucimobilis* and *L. varians* showed correlations. In the water samples, the total bacterial counts were correlated with *M. luteus* and *S. paucimobilis*, although these two species were not most abundant in the samples. In the air samples, *S. haemolyticus*, *M. organophilum*, *L. varians*, and *B. thuringiensis* were correlated with the total bacterial count, and *S. haemolyticus*, *M. organophilum*, and *L. varians* were abundant in all of the samples.

3.3 Methylotrophic and keratin degradation activity

Methylobacterium organophilum was the only bacterium that is methylotrophic and could use methanol as its sole carbon source. *L. varians* grew more than 5 $\log_{10}$ during the 4-day incubation with skin cells as the sole nitrogen source (Table 4). *S. haemolyticus* and *B. thuringiensis* also grew well in the medium (3.77 and 4.14 $\log_{10}$ changes, respectively). These bacteria showed strong keratinase activity and ammonium production, which implies that keratins were degraded and nutrients were released to support bacterial growth. In terms of keratinase activity per extracellular protein measured, *S. haemolyticus* and *B. thuringiensis* doubled the yield of *L. varians*. Considering the population sizes of these bacteria, *B. thuringiensis* produced the most net ammonium (4.61 mg/10^{14} CFU), almost twice that of *S. haemolyticus* and six times that of *L. varians*. In general, keratinase was about 40–65% more effective at 20 °C than at 30 and 4 °C. The low temperature condition (4 °C) did not stop the enzyme activity, which implies that if keratinase is present in the air-cooling unit, it will function properly across different phases of the air-cooling cycle. Figure 1 shows that keratinolytic activity was higher in the units with odor complaints than that without.

4 Discussion

4.1 Hypothesis 1: Units with and without odor complaints have different microbial communities

Methylobacterium organophilum and *S. haemolyticus* released into the air before and during the spraying process are the significant bacterial species that contributed to the dissimilarity of the microbial communities in the units with and without odor complaints. Previous studies have reported that

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Table 3 Interspecies correlation in water and air samples

Species	Water	Air before spray	Air during spray
S. haemolyticus	No correlation	*M. organophilum* ($r = 0.878$, $p = 0.004$) *B. thuringiensis* ($r = 0.770$, $p = 0.025$) Total bacteria ($r = 0.970$, $p = 0.000$)	*M. organophilum* ($r = 0.814$, $p = 0.014$) *B. thuringiensis* ($r = 0.921$, $p = 0.001$) *L. varians* ($r = 0.735$, $p = 0.038$) Total bacteria ($r = 0.976$, $p = 0.000$)
M. organophilum	*B. thuringiensis* ($r = 0.733$, $p = 0.025$) Total fungi ($r = 0.817$, $p = 0.007$)	*S. haemolyticus* ($r = 0.878$, $p = 0.004$) *B. thuringiensis* ($r = 0.845$, $p = 0.008$) Total bacteria ($r = 0.834$, $p = 0.010$)	*S. haemolyticus* ($r = 0.814$, $p = 0.014$) *B. thuringiensis* ($r = 0.722$, $p = 0.043$) Total bacteria ($r = 0.850$, $p = 0.007$)
M. luteus	Total bacteria ($r = 0.895$, $p = 0.001$)	No correlation	No correlation
B. thuringiensis	*M. organophilum* ($r = 0.733$, $p = 0.025$) Total fungi ($r = 0.667$, $p = 0.050$)	*S. haemolyticus* ($r = 0.770$, $p = 0.025$) *M. organophilum* ($r = 0.845$, $p = 0.008$) Total bacteria ($r = 0.823$, $p = 0.012$) *P. chryosgenum* ($r = 0.794$, $p = 0.019$) *A. fumigatus* ($r = 0.713$, $p = 0.047$) Total fungi ($r = 0.733$, $p = 0.039$)	*S. haemolyticus* ($r = 0.921$, $p = 0.001$) *M. organophilum* ($r = 0.722$, $p = 0.043$) Total bacteria ($r = 0.872$, $p = 0.005$) *A. fumigatus* ($r = -0.716$, $p = 0.046$)
S. paucimobilis	*L. varians* ($r = 0.711$, $p = 0.032$) Total bacteria ($r = 0.750$, $p = 0.020$)	*L. varians* ($r = 0.730$, $p = 0.040$)	No correlation
S. epidermidis	No data	No correlation (limited sample size)	No correlation (limited sample size)
L. varians	*S. paucimobilis* ($r = 0.711$, $p = 0.032$)	*S. paucimobilis* ($r = 0.730$, $p = 0.040$)	*S. haemolyticus* ($r = 0.735$, $p = 0.038$) Total bacteria ($r = 0.711$, $p = 0.048$) *P. uncinulatum* ($r = -0.897$, $p = 0.003$)
P. chryosgenum	No correlation	*B. thuringiensis* ($r = 0.794$, $p = 0.019$) *A. fumigatus* ($r = 0.790$, $p = 0.020$) Total fungi ($r = 0.871$, $p = 0.005$)	*A. fumigatus* ($r = 0.747$, $p = 0.033$) Total fungi ($r = 0.934$, $p = 0.001$)
M. ramosissimus	No correlation	*A. fumigatus* ($r = 0.895$, $p = 0.003$) Total fungi ($r = 0.785$, $p = 0.021$)	Total fungi ($r = 0.755$, $p = 0.031$)

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Table 3 continued

Species	Water	Air before spray	Air during spray
A. fumigatus	No correlation	*B. thuringiensis* ($r = 0.713$, $p = 0.047$) *P. chryosgenum* ($r = 0.790$, $p = 0.020$) *M. ramosissimus* ($r = 0.895$, $p = 0.003$) Total fungi ($r = 0.954$, $p = 0.000$)	*B. thuringiensis* ($r = -0.716$, $p = 0.046$) *P. chryosgenum* ($r = 0.747$, $p = 0.033$) Total fungi ($r = 0.814$, $p = 0.014$)
P. uncinulatum	No correlation	No correlation	*L. varians* ($r = -0.897$, $p = 0.003$)

methylobacteria are commonly found on aluminum evaporator fins in automobile ACSs, regardless of whether odor is reported, because of its resistance to desiccation (Simmons et al. 1999; Yano et al. 2013). Further studies by Rose et al. (2000) showed that *Methylobacterium mesophilicum* biofilms produced mainly dimethyl disulfide on the aluminum fins, whereas those in combination with *Penicillium viridicatum* produced offensive smelling alcohols and esters. Some methylobacteria can live on the human foot, scalp, and nasal cavity by feeding on methylated sulfur compounds from the degradation of amino acids and produce body odor (Anesti et al. 2004; Uy et al. 2013). Methylobacteria can consume single-carbon and multicarbon compounds that have no C–C bond, which may be another competitive advantage that allows them to propagate in the air-cooling unit. Although fungal propagation is commonly associated with surface contamination and odor production, fungal communities did not show a correlation with odor complaints in this study. *S. haemolyticus* was associated with odorous units. This species has never been reported in ACSs. *S. haemolyticus* is part of the human skin flora that is notorious for producing some of the most offensive sulfurous odor and can form biofilms on implanted medical devices, which makes it an important nosocomial pathogen (Fredheim et al. 2009; James et al. 2012). In general, *Staphylococcus* isolates from the human body contribute to the production of short-chain volatile fatty acids, e.g., isovaleric acids from L-leucine, a branched aliphatic amino acid. Isovaleric acids are the typical malodorants associated with the acidic smell of body odor (James et al. 2012). Moreover, *S. haemolyticus* plays a role in the production of a thioalcohol 3-methyl-3-mercaptohexan-1-ol malodorant by cleaving its precursors, *S*-hydroxyalkyl-L-cysteinylglycine conjugates (James et al. 2012). Although both species can produce malodorants associated with body odor, these malodorants are not urine-like. Therefore, the next question is whether ammonium is present in the unit to justify the potential of releasing ammonia from the microbial community.

4.2 Hypothesis 2: Presence of keratins and odor production in the units

The urine-like odor sensation may come from ammonia. The toxicity of ammonia has been reviewed by the US Environmental Protection Agency (2013). The inhalation of several ppm of ammonia (e.g., >5 ppm) is reported to cause eye discomfort, headache, dizziness, and a feeling of intoxication. It is therefore an important issue that needs to be addressed. Ammonia can be released directly by some microbes via degradation of organic nitrogen, such as amino acids and urea by deamination and production of urease, respectively (Kada et al. 2008). Bacterial degradation of organic nitrogen or fixing of atmospheric nitrogen to ammonium, which is then converted to ammonia in alkali conditions (e.g., at $pH > 8$), is also a possible mechanism. Alkali conditions can be created when free amino acids are deaminated. Because ammonia can be converted from ammonium in water, and the directly produced ammonia can dissolve in water to become ammonium, our approach is to test for the presence of ammonium in the unit and to determine whether its level correlates with the odor complaints. All units had measurable amounts of ammonium in the eluted water samples, but the average levels did not

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Table 4 Keratin degradation and protein and ammonium production

Bacteria	Log_{10} increase[@]/CFU/mL[#]	Ammonium[@] (mg/100 mL) (std)	Ammonium/CFU (mg/10^{14} CFU)	Protein[@] (mg/100 mL) (std)	Keratinase activity[$] (std)			Keratinase at 20 °C/protein
					30 °C	20 °C	4 °C	
S. haemolyticus	3.77 2.77×10^9	0.007 (0.002)	2.52	8.43 (0.74)	0.061^b (0.003)	0.102^a (0.004)	0.063^b (0.003)	0.012
M. organophilum	1.14 4.78×10^6	0.001 (0.000)	N.A.	0.81 (0.08)	0.004 (0.003)	0.001 (0.001)	0.001 (0.001)	N.A.
M. luteus	1.16 6.44×10^6	0.000 (0.000)	N.A.	0.29 (0.15)	0.004 (0.002)	0.001 (0.001)	0.001 (0.000)	N.A.
B. thuringiensis	4.14 5.64×10^9	0.026 (0.002)	4.61	9.91 (1.33)	0.082^b (0.002)	0.126^a (0.006)	0.091^b (0.008)	0.013
S. paucimobilis	1.16 7.33×10^6	0.001 (0.000)	N.A.	0.57 (0.14)	0.004 (0.002)	0.001 (0.000)	0.001 (0.001)	N.A.
S. epidermidis	1.08 5.11×10^6	0.001 (0.000)	N.A.	0.48 (0.17)	0.007 (0.003)	0.001 (0.000)	0.002 (0.001)	N.A.
L. varians	5.08 6.64×10^{10}	0.058 (0.001)	0.873	31.86 (0.43)	0.117^b (0.007)	0.203^a (0.007)	0.124^b (0.006)	0.006

[@] Experimental conditions: 4-day incubation with skin scales at 30 °C

[#] Standard deviations of the cell counts (CFU/mL) are all under 5.5% of the mean

[$] Experimental conditions: Keratin azure added into the filtrates of the 4-day cultures; Keratinase activity measured at OD_{595} after 12 days of incubation at different temperatures

[a,b] Statistical significance between means ($p < 0.05$)

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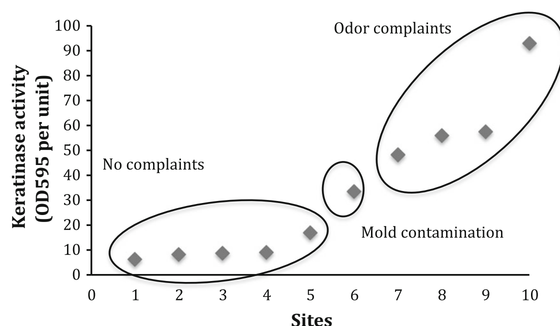

Fig. 1 Keratinase activity in water samples eluted from air-cooling units. Sites: *1* activity room 2; *2* lift lobby 2; *3* project room 2; *4* open-plan office 2; *5* activity room 1; *6* vacant office; *7* project room 1; *8* open-plan office 1; *9* pantry; *10* lift lobby 1

differ significantly between units with and without odor complaints. Further studies are needed to investigate the ammonia emission mechanism.

Few studies have investigated the contents of dirt, dust, and litter that provide nutrients to microbes in air-cooling units (Simmons et al. 1999; Beko et al. 2006). In this study, the indoor environments were not directly exposed to the outdoors and were regularly vacuumed and surface cleaned. The presence of keratinase, induced by keratins, supports the hypothesis that keratins, likely come from human skin, may accumulate in the air-cooling units. Our study is the first to report keratinase activity in air-cooling units, and its activity level was higher in units with odors. Human skin cells, which are naturally and continuously released into the air, could be degraded by microbes in the units and provide a diet high in protein and nitrogen for the production of ammonium as shown in our laboratory experiments. *S. haemolyticus*, *B. thuringiensis*, and *L. varians* multiplied more than 3 $\log_{10}$ and produced a high level of ammonium in the skin scale medium. The microbial ability to degrade keratins justifies the production of various malodorants, such as short-chain volatile fatty acids from amino acids. The unique nutritional modes of keratinolytic and methylotrophic bacteria may convert the nutrient poor environment to become nutrient rich and help the propagation of microorganisms in the seemingly 'clean' air-cooling unit. The skin scales also contain other readily available nutrients, such as free amino acids and other skin excretions, which explain the increase in the population sizes of other bacteria to one $\log_{10}$ (James et al. 2012).

If skin cells are one of the main sources of nutrients, other non-keratinolytic bacteria will rely on these bacteria to grow and show some population correlation in the field samples. Our results support this point; the population of *M. organophilum* (non-keratinolytic) was correlated with *B. thuringiensis* in water samples and with *S. haemolyticus* and *B. thuringiensis* in air samples; *S. paucimobilis* (non-keratinolytic) was correlated with *L. varians* in water and background air samples. The only exception was *M. luteus* (non-keratinolytic), which was not correlated with any single species. However, interestingly, in the water samples, *M. luteus* and *S. paucimobilis* were correlated with the total bacterial counts, although these two species were not most abundant in the samples. The lack of a significant difference in the counts of *M. luteus* in the samples collected before and during the spraying process suggests that *M. luteus* was not a dominant species on the evaporator fin. *M. luteus* may have inhabited the drain pan and relied on the metabolites from other self-supplied bacteria as a food source, and thus correlated with the total bacterial count.

5 Conclusions

This study reveals that viable airborne microbial counts commonly used in IAQ assessment can help differentiate air-cooling units with and without complaints of urine-like and body odors. This could help IAQ inspectors to identify potential odor-causing units for cleaning. Moreover, keratins in human skin scales may be degraded in the units and provided nutrients for microbial growth and odor production.

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References

Anesti, V., Vohra, J., Goonetilleka, S., McDonald, I. R., Straubler, B., Stackebrandt, E., et al. (2004). Molecular detection and isolation of facultatively methylotrophic bacteria, including *Methylobacterium podarium* sp. nov., from the human foot microflora. *Environmental Microbiology, 6*(8), 820–830.

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Barkat, S., Le Berre, E., Coureaud, G., Sicard, G., & Thomas-Danguin, T. (2012). Perceptual blending in odor mixtures depends on the nature of odorants and human olfactory expertise. *Chemical Senses, 37*(2), 159–166.

Beko, G., Halas, O., Clausen, G., & Weschler, C. J. (2006). Initial studies of oxidation processes on filter surfaces and their impact on perceived air quality. *Indoor Air, 16*(1), 56–64.

Bluyssen, P. M., Cox, C., Seppanen, O., Fernandes, E. O., Clausen, G., Muller, B., et al. (2003). Why, when and how do HVAC-systems pollute the indoor environment and what to do about it? The European AIRLESS project. *Building and Environment, 38*(2), 209–225.

Bressollier, P., Letourneau, F., Urdaci, M., & Verneuil, B. (1999). Purification and characterization of a keratinolytic serine proteinase from *Streptomyces albidoflavus*. *Applied Environmental Microbiology, 65*(6), 2570–2576.

Clarke, K. R., & Warwick, R. M. (2001). *Change in marine communities: An approach to statistical analysis and interpretation*. Plymouth: Primer-E.

Fredheim, E. G. A., Klingenberg, C., Rohde, H., Frankenberger, S., Gaustad, R., Flaegstad, T., et al. (2009). Biofilm formation by *Staphylococcus haemolyticus*. *Journal of Clinical Microbiology, 47*(4), 1172–1180.

Hong Kong Environmental Protection Department. (2003). Indoor Air Quality Management Group. A guide on indoor air quality certification scheme for offices and public places. The Government of the Hong Kong Special Administrative Region.

Hugenholtz, P., & Fuerst, J. A. (1992). Heterotrophic bacteria in an air-handling system. *Applied Environmental Microbiology, 58*(12), 3914–3920.

James, A. G., Austin, C. J., Cox, D. S., Taylor, D., & Calvert, R. (2012). Microbiological and biochemical origins of human axillary odor. *FEMS Microbiology Ecology, 83*(3), 527–540.

Kada, S., Yabusaki, M., Kaga, T., Ashida, H., & Yoshida, K. I. (2008). Identification of two major ammonia-releasing reactions involved in secondary natto fermentation. *Bioscience, Biotechnology, and Biochemistry, 72*(7), 1869–1876.

Kosonen, R., & Tan, F. (2004). The effect of perceived indoor air quality on productivity loss. *Energy and Buildings, 36*(10), 981–986.

Rose, L. J., Simmons, R. B., Crow, S. A., & Ahearn, D. G. (2000). Volatile organic compounds associated with microbial growth in automobile air conditioning systems. *Current Microbiology, 41*(3), 206–209.

Simmons, R. B., Rose, L. J., Crow, S. A., & Ahearn, D. G. (1999). The occurrence and persistence of mixed biofilms in automobile air conditioning systems. *Current Microbiology, 39*(3), 141–145.

Tananuvat, N., Salakthuantee, K., Vanittanakom, N., Pongpom, M., & Ausayakhun, S. (2012). Prospective comparison between conventional microbial work-up vs PCR in the diagnosis of fungal keratitis. *Eye, 26*(10), 1337–1343.

U.S. EPA. (2013). IRIS toxicological review of ammonia (Revised external review draft). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-13/139a.

Uy, M. M., Uy, J., Carvajal, T. M., Castro, C. Z. R., Ho, H. T., & Lee, A. C. (2013). Pink pigmented facultative methylotrophic (PPFM) bacteria isolated from the hair scalp and nasal cavity. *Phillippine Journal of Systematic Biology, 7*, 13–21.

Yano, T., Kubota, H., Hanai, J., Hitomi, J., & Tokuda, H. (2013). Stress tolerance of *Methylobacterium* biofilms in bathrooms. *Microbes and Environment, 28*(1), 87–95.