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Skin squames contribute to ammonia and volatile fatty acid production from bacteria colonizing in air-cooling units with odor complaints

T. W. Ng  
*Hong Kong Baptist University*

P. Y. Chan  
*Hong Kong Baptist University*

T. T. Chan  
*Hong Kong Baptist University*

H. Wu  
*Hong Kong Baptist University*

K. M. Lai  
*Hong Kong Baptist University, laikaman@hkbu.edu.hk*

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1 **Skin squames contribute to ammonia and volatile fatty acid**  
2 **production from bacteria colonizing in air-cooling units with odor**  
3 **complaints**

4  
5 **Tsz Wai NG, Pui Ying CHAN, Tsz Ting CHAN, Haoxiang WU, Ka Man LAI\***

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7 *Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special*  
8 *Administrative Region.*

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14 \*Corresponding authors. Tel.: +852 3411 5838; e-mail: laikaman@hkbu.edu.hk.

15 **Abstract**

16 One of the most notable Indoor Air Quality problems is odour emission. This study  
17 investigated the potential contribution of skin squames to the production of ammonia (NH<sub>3</sub>)  
18 and volatile organic acids (VFAs) by 7 bacteria isolated from air-cooling (AC) units with  
19 complaints of urine and body odors. Our previous study showed that keratinolytic activity is  
20 higher in AC units with odor complaints than those without. In the offices where these units  
21 are located, the most likely source of keratins is from human skin squames. Most bacteria can  
22 produce NH<sub>3</sub> and VFAs in the skin squame culture. Some correlations between the levels of  
23 NH<sub>3</sub>, NH<sub>4</sub><sup>+</sup>, VFAs, and keratinolytic activity were found. The odour production pathway with  
24 skin squames was proposed. *Staphylococcus haemolyticus* was abundant in the AC units with  
25 odour problems and had a high level of keratinolytic activity in addition to odour production.  
26 For long-term odor control, it is important to reduce the level of skin squames entering the  
27 AC units.

28 **Keyword: air-conditioning, ammonia, building microbiology, Indoor Air Quality, odour**  
29 **control, skin squames, volatile fatty acids**

## 30 **Practical Implications**

31 This study demonstrates the potential contribution of skin squames to the production of  
32 ammonia (NH<sub>3</sub>) and volatile organic acids (VFAs) from bacteria colonising in air-cooling  
33 (AC) units with complaints of urine and body odors. Understanding the source and  
34 mechanism of odour production helps to formulate the control measures. For long-term odour  
35 control, it is important to reduce the level of skin squames entering the AC units, such as by  
36 improving the filter quality of the air-handling unit. Several studies have reported human  
37 occupancy as a source of indoor airborne bacteria and the presence of skin flora in the air.  
38 This study demonstrates their potential active role in deteriorating Indoor Air Quality by  
39 colonizing in AC units.

## 40 **Introduction**

41 Air-conditioning systems (ACSs) are necessary to maintain the comfort and air quality of  
42 indoor environments in many places. However, the release of unpleasant smells from ACSs  
43 deteriorates the Indoor Air Quality and reduces the occupant's productivity.<sup>1</sup> There were  
44 complaints of urine and body-odor like smells from some individual air-cooling AC units at  
45 our university campus and in some cases, the smell returned within months even after cleaning  
46 the system.<sup>2</sup> Investigating odor problems from ACSs is not new in building research, and the  
47 problem is mainly due to poor maintenance and dirty environments.<sup>1</sup> The unusual situation of  
48 these relatively enclosed strong smelling offices without any other known source of odour, dust,  
49 or microbial contamination, and with daily cleaning, prompted this investigation into the cause  
50 of the odor problems. Once the source and mechanism of odor production are revealed,  
51 strategic and effective measures can be devised to mitigate this problem.

52 Our previous study found that the level of keratinolytic activity - ability to break down  
53 keratins such as through the disulfide bond (-S-S-) to release proteins for further degradation -  
54 in AC units is positively correlated to odor complaints.<sup>2-4</sup> This provides a clue to the link  
55 between odor production and keratins. The major source of keratins in the indoor environments  
56 that we investigated likely comes from the skin squames from occupants. Squames refer to the  
57 terminally differentiated, enucleated keratinocytes, which are located at the top layer of the  
58 epidermis, namely the stratum corneum. Squames consist of keratin fibrils and cross-linked

59 cornified envelopes embedded in lipid bilayers, forming the ‘bricks and mortar’ of the  
60 epidermis.<sup>5</sup> Squames are constantly shed in the process of desquamation at a rate which  
61 balances the production of corneocytes so that a constant thickness of stratum corneum can  
62 be maintained. Millions of dead skin cells are shed off from the skin every day.<sup>6</sup> Although the  
63 size of skin squames is generally larger than 10  $\mu\text{m}$ ,<sup>6</sup> making it easy to settle soon after  
64 aerosolization, it may be possible that some smaller skin squames which have shed are  
65 brought into the AC units if they are not captured by the filter of the AC unit.

66 This study hypothesizes that skin squames containing keratins and other constituents may  
67 serve as nutrients for bacterial growth and odor production in AC units. Acerbi et al.<sup>7</sup> reported  
68 an ecological succession of microbial communities in AC units. Hugenholtz et al.<sup>8</sup> and  
69 Simmons et al.<sup>9</sup> analyzed the microbial properties and the odour production process, different  
70 from the one proposed in this study. All these reports justified the presence and growth of  
71 microbes in AC units and the potential of producing odor from microbial activity. Grice and  
72 Segre<sup>10</sup> reviewed various aspects of the skin microbiome. Our skin provides a diverse habitat  
73 (e.g. aerobic and anaerobic; wet and dry conditions) and nutrients (e.g. lipid secretion and  
74 amino acids in sweat) to support a wide range of microorganisms. Several studies reported  
75 the bacterial odor production pathways in the human body.<sup>5,11-12</sup> Propionibacteria,  
76 Staphylococci and some other anaerobic bacteria, *Anaerococcus* spp. ferment glycerol and  
77 lactic acid to odorous short chain volatile fatty acids (VFAs) such as acetic and propionic

78 acid. Odor production can also happen under aerobic conditions. For instance, corynebacteria  
79 generate VFAs from lipid substrates to produce axillary odour.<sup>11</sup> *Staphylococcus* spp. and  
80 *Bacillus* spp. are capable of converting branched aliphatic amino acids, leucine to produce a  
81 highly odorous iso-valeric acid, a typical foot odourant.<sup>12</sup> Skin microbiology, body odor, and  
82 methylotrophic bacteria were reviewed by Kelly and Wood.<sup>5</sup> Not only do the excreted from the  
83 skin such as amino acids in eccrine sweat and apocrine secretion directly provide nutrients  
84 and parental sources for different odor production, keratinizing epidermis can also release  
85 amino acids after further bacterial proteolysis.<sup>11</sup> VFAs, such as hexanoic acid, isovaleric acid  
86 and isobutyric acid, are widely recognized as being responsible for the unpleasant smells  
87 from humans.<sup>5</sup> Ara et al.<sup>12</sup> stated that the odor of isovaleric acid could be perceived at about  
88 0.01 ppm.

89 Urine-like smells are commonly associated with ammonia (NH<sub>3</sub>), which is a waste  
90 product occurring from the breakdown of some amino acids. Ammonium (NH<sub>4</sub><sup>+</sup>) was  
91 detected in the AC units, which further supports our hypothesis that skin squames are  
92 consumed by some bacteria inhabiting the cooling coil aluminum fin environment that  
93 produce amino acids and then NH<sub>3</sub>.<sup>2</sup> NH<sub>3</sub> is a pungent gas and has an odor threshold of 5 ppm.  
94 High levels of NH<sub>3</sub> (24 ppm) can cause nasal irritation.<sup>13</sup>

95 No study has investigated whether skin squames, which are naturally present in occupied  
96 environments, can be used by bacteria in AC units to produce odor.<sup>1,9</sup> This study aims to test

97 this hypothesis and investigate whether the dominant bacteria in AC units with odor problems  
98 (*Staphylococcus haemolyticus* and *Methylobacterium organophilum*) also produce the highest  
99 level of odor. Finally, this study proposes long-term control measures based on our new  
100 findings.

101

## 102 **Materials and Methods**

### 103 *Preparation of Skin Squames*

104 Foot skin squames were collected from healthy donors without any known skin disease  
105 using foot files. A series of tests was conducted to design a sanitization method that can  
106 inhibit the growth of foot flora when the skin squames were used in the culture with the  
107 bacteria isolated from AC units. Finally, we found that exposure of the skin squames to  
108 ultraviolet light for 24 hours and then heated in the oven at 50°C for 3 days could inhibit  
109 microbial contamination from the skin squames. The sanitized skin squames were stored in a  
110 desiccator before use.

111

### 112 *Bacterial Culture*

113 The bacteria tested were *S. haemolyticus*, *M. organophilum*, *Micrococcus luteus*, *Bacillus*  
114 *thuringiensis*, *Sphingomonas paucimobilis*, *Staphylococcus epidermidis*, and *Lysinibacillus*  
115 *varians*.<sup>2</sup> These bacteria were isolated using culture-based methods under aerobic condition in  
116 our previous study.<sup>2</sup> When AC units are in operation, a high flow rate of air is likely to create



117 an aerobic condition for the microbial community around the cooling coil fin environment.  
118 These bacteria were incubated to the stationary phase in tryptic soya broth (TSB) at 30°C and  
119 150 rpm for 24 hours (72 hours for *M. organophilum* to reach the stationary phase). The  
120 bacteria were harvested by centrifugation and then washed 3 times with phosphate buffered  
121 saline (PBS, pH 7.4), and finally resuspended in PBS.

122

### 123 *Experimental Set-up*

124 *Odour production potential.* Twenty milligrams of skin squames and 5 mL of sterilized  
125 deionized water were added to a 250-mL conical flask to reflect the substrate condition that  
126 occurs in the cooling coil fin environment in our investigation. Using 20 mg of skin squames in  
127 the culture was adequate for the comparison of the odor production property of the bacteria as  
128 shown in our previous study.<sup>2</sup> Using deionized water rather than a PBS buffer in the culture was  
129 intentional as a way to mimic the water source of the fin environment from condensation. Half  
130 of 1 mL of the freshly prepared bacteria at 10<sup>8</sup> CFU/mL was inoculated into the substrate  
131 mixture. This cell density was related to the carrying capacity of the bacteria under this  
132 experimental condition determined using growth studies. The bacterial levels were maintained  
133 during the experiment. Using the same cell density in each bacterial culture allows an equal  
134 comparison between the bacteria. The bacterial culture was incubated at 30°C at 150 rpm for 3  
135 days. During the experiment, the levels of NH<sub>3</sub> (detection limit: 0.2 ppm) and VFAs (detection

136 limit: 1 ppm) in the air were determined daily by Kitagawa precision gas detection tubes  
137 (Komyo Rikagaku Kogyo K.K., Kanagawa, Japan). After 3 days, the  $\text{NH}_4^+$  concentration,  
138 VFAs concentration, urease activity, keratinolytic activity and pH of the bacterial culture were  
139 determined as well. The experiments were conducted in triplicates with control setups which  
140 had no bacteria added. Since bacteria are not able to grow in the absence of nutrients (e.g.  
141 carbon and nitrogen sources), control setups with bacteria and water only are not required.  
142 Bacterial growth in the controls was checked using the plate-counting method with tryptic  
143 soya agar incubated at 30°C for 2 days. No bacteria were found on the agar plates.

144

145 *Temperature effect.* The effect of incubation temperature (10, 15 and 20°C) on  $\text{NH}_3$  and VFAs  
146 production was examined because the temperature of the cooling coil fin varies during the  
147 temperature control cycle. According to our engineers, the general cooling coil temperature is  
148 ~ 7°C. After reaching the set room temperature, the cooling operation turns off automatically,  
149 and the cooling coil fin temperature rises with the room temperature immediately until the  
150 next cooling cycle. *S. haemolyticus*, *M. organophilum* and *B. thuringiensis* were examined  
151 because *S. haemolyticus* and *M. organophilum* were abundant in the AC units with odor  
152 complaints,<sup>2</sup> while *B. thuringiensis* showed the highest potential for  $\text{NH}_3$  and VFAs  
153 production. The experiments were conducted in triplicates.

154

155 *Analytical Methods*

156 The  $\text{NH}_4^+$  concentration and keratinolytic activity were determined by the indophenol  
157 blue method<sup>14</sup> and keratinolytic activity assay with keratin azure (Sigma, St. Louis, MO,  
158 USA),<sup>15</sup> respectively, as mentioned in our previous study.<sup>2</sup> The pH of the solution was  
159 measured by a pH meter. Urease activity was measured by a urease assay kit (Sigma)  
160 according to the protocol suggested by the manufacturer.

161 The profile of VFAs and concentration of the bacterial culture were measured using  
162 high-performance liquid chromatography equipped with an organic acid column (Aminex  
163 HPX-87H column, Bio-rad, Hercules, CA, USA). The column was operated at 60°C with  
164 0.1% phosphoric acid at 1 mL/min as eluent. A UV detector at 210 nm was used to detect the  
165 analysts. Different concentrations of the VFAs standard solution (Supelco, Bellefonte, PA,  
166 USA) were used to construct a standard curve to determine the unknown concentration of  
167 VFAs in the samples. Formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid,  
168 isovaleric acid, valeric acid, isocaproic acid, and hexanoic acid were analyzed in this study.

169

170 *Statistical analysis*

171 The change in the  $\text{NH}_3$  level between the bacterial cultures over the study period was  
172 compared using repeated measures analysis of variance (ANOVA) (SPSS v. 23, IBM Corp.,  
173 Armonk, NY, USA). The  $\text{NH}_4^+$  level, VFAs level, keratinolytic activity and urease activity

174 between the bacterial cultures on day 3 were compared using one-way ANOVA (SPSS v. 23).  
175 Grouping was conducted using post-hoc test Duncan analysis. Differences between means  
176 with  $P$ -values lower than .05 ( $P < .05$ ) were regarded as statistically significant. The  
177 correlation between different parameters ( $\text{NH}_3$ ,  $\text{NH}_4^+$ , keratinolytic activity, urease activity  
178 and VFAs) in each culture was analyzed using Pearson's correlation analysis (SPSS v. 23).  
179 Correlation with  $P$ -values lower than .05 ( $P < .05$ ) was regarded as statistically significant.

180

## 181 **Results and discussion**

### 182 *Odor production potential of the bacteria*

183 *Bacillus thuringiensis* showed the fastest rate in the production of  $\text{NH}_3$ ; more than 20  
184 ppm of  $\text{NH}_3$  was emitted within a day of incubation (Figure 1). After 3 days, *B. thuringiensis*  
185 emitted the highest level of  $\text{NH}_3$  (35 ppm), followed by *S. haemolyticus* (34.5 ppm), *S.*  
186 *paucimobilis* (33 ppm), *S. epidermidis* (33 ppm) and *M. luteus* (24 ppm).  $\text{NH}_3$  production by  
187 *M. organophilum* (13 ppm) and *L. varians* (4.5 ppm) was limited and statistically similar to  
188 the control. Although no bacteria were found on the agar plates from the control culture, skin  
189 squames may contain  $\text{NH}_4^+$  that can convert to  $\text{NH}_3$  during incubation. Moreover, slight  
190 biological activity from bacteria and enzymes may also contribute to the detectable  $\text{NH}_3$   
191 levels in the control setups.  $\text{NH}_4^+$  was detected in the bacterial culture after 3 days of  
192 incubation, but the levels were largely similar between cultures, except for *L. varians*, which

193 had a similar  $\text{NH}_4^+$  level to the control (Figure 2A). No VFAs were detected in the air during  
194 the whole period of incubation but VFAs were detected in the bacterial culture (Figure 2B).  
195 Hexanoic acid, associated with axillary odor, was widely detected in all the cultures, while  
196 the controls had a relatively high level of propionic acid. Other than acetic acid, propionic  
197 acid is the second highest level fatty acid in sweat odor.<sup>11</sup> Acetic acid, as one of the simplest  
198 carboxylic acids involved in the metabolism of carbohydrates and fats, may be consumed by  
199 skin bacteria more readily than other VFAs. This may explain a higher fraction of propionic  
200 acid detected in the skin squame control. *B. thuringiensis* produced the highest amount of  
201 total VFAs. For the dominant bacteria in AC units with odour problems, *S. haemolyticus*  
202 produced a mixture of VFAs while *M. organophilum* mainly produced hexanoic acid. *S.*  
203 *haemolyticus* is a facultative anaerobe, and *M. organophilum* is an obligate aerobe and a  
204 facultative methylotroph.<sup>5,16</sup> The equilibrium between the chemicals in the air and the solution  
205 is dependent on pH.  $\text{NH}_3$  as a weak base emits to the air from the conjugate acid,  $\text{NH}_4^+$  at  
206 high pH levels (acid dissociation starts at  $\text{pH} > 7$ ) because  $\text{NH}_4^+$  has a pKa of 9.25, while  
207 VFAs have pKa in the range of 4.76-4.88. According to our measurement, the skin squame  
208 control had a pH of about 8.2, while the pH of the bacterial solution after 3 days incubation  
209 ranged from 8.27 to 8.44. This explains the non-detectable levels of VFAs in the air even  
210 though they are produced by the bacteria in this study. In summary, these results support our  
211 hypothesis that most bacteria isolated from AC units with odor problems were able to

212 produce both NH<sub>3</sub> and VFAs, our targeted odors with skin squames, but *S. haemolyticus* and  
213 *M. organophilum* were not the highest odor producers. In the other words, some factors in the  
214 AC unit may favour the population of *S. haemolyticus* and *M. organophilum*, and these  
215 factors also facilitate the odor production by *S. haemolyticus*. As a result, *S. haemolyticus*, but  
216 not *B. thuringiensis*, was associated with the AC units with odor complaints. *S. haemolyticus*  
217 is a common skin flora, which may continuously invade the cooling coil fin environment with  
218 the skin squames and take the advantage of consuming the nutrients and, hence, produce the  
219 odor. *Methylobacterium spp.* are common bacteria found in AC units due to their general  
220 dry-resistance, but their role in producing NH<sub>3</sub> and VFAs is not significant in the single  
221 culture condition.<sup>8,9,17,18</sup>

222

### 223 *Odor production pathway*

224 The above results demonstrate that most bacteria inhabiting AC units can utilize skin  
225 squames to produce NH<sub>3</sub> and VFAs. This part of the study aimed to investigate the pathway  
226 of the odor production (Figure 3). Skin squames contain lipids, keratins and other constituents  
227 such as waste products from skin excretion, which can become a source of the odor. Not all  
228 bacteria can produce keratinases (a group of extracellular enzymes that performs the  
229 keratinolytic activity), and the production of keratinases is only induced by the presence of  
230 keratins.<sup>3,4</sup> The ability of producing keratinases is an important step to increase the nutrient

231 level in the bacteria culture, and the nutrient provided by keratins is protein. Figure 4 shows  
232 the keratinolytic activity of the bacteria. *B. thuringiensis* had the highest activity followed by  
233 *S. haemolyticus*. Although *M. organophilum* was also dominant in the AC units with odor  
234 problems, its keratinolytic activity was low. After a series of proteolysis by extracellular  
235 proteases and peptidases, proteins are broken down into various peptides and then amino  
236 acids which can be taken up by bacteria (Figure 3). Through the general process of  
237 deamination, the amino group ( $-NH_2$ ) of amino acids ( $H_2NCHR_1COOH$ ) is removed to form  
238  $NH_3$  via hydrolysis, and then,  $NH_3$  reacts with  $H_2O$  to become  $NH_4^+$  and  $OH^-$ , which can be  
239 excreted from the bacterial cell.<sup>19</sup> The degradation of amino acids in the bacterial culture  
240 increases the level of  $NH_4^+$  and  $OH^-$  (increase the pH value) and, ultimately,  $NH_3$  is released  
241 from  $NH_4^+$  at high pH conditions. This alkaline condition also favors keratinolysis because of  
242 the increased accessibility of cysteine residues for keratinase action.<sup>3,4</sup> Another more direct  
243 pathway to release  $NH_3$  is using ureases to break down urea, which may be present in the skin  
244 squames as a waste metabolite or produced by amino acids; arginase cleaves arginine to form  
245 urea (Figure 3).<sup>20</sup> Therefore, bacteria that had the highest urease activity (i.e. *B. thuringiensis*)  
246 can have extra  $NH_3$  production from degrading urea (Figure 5). In terms of VFAs production,  
247 various branched amino acids, lipids and long-chain fatty acids are involved in their synthesis  
248 (Figure 3).<sup>12</sup> For example, leucine degradation by *Bacillus* spp. produces isovaleric acid and  
249 isobutyric acid.<sup>12</sup> James et al also reported the conversion of isobutyric acid and isovaleric

250 acid from valine and leucine by *Staphylococcus* spp. Long-chain fatty acids are degraded to  
251 short-chain fatty acids by *Micrococcus* spp.<sup>12</sup> The corollary is that different nutrient (e.g.  
252 amino acids and lipids) profiles of the skin squames and bacteria are likely to contribute to  
253 different VFAs emissions (Figure 2).

254 Our previous finding showed that the high abundance of *S. haemolyticus* and *M.*  
255 *organophilum* were correlated to odor production. However, the NH<sub>3</sub> production and  
256 keratinolytic activity of *M. organophilum* were relatively low. This result led to the  
257 investigation into whether the coculture of these dominant bacteria in AC units with odor  
258 problems would increase the NH<sub>3</sub> production because *S. haemolyticus* helps raise the amino  
259 acid content through the high keratinolytic activity, which *M. organophilum* may benefit from  
260 to produce NH<sub>3</sub>. This coculture condition (inoculation with 0.25 mL of 10<sup>8</sup> cfu/mL of each  
261 bacterium, half of the inoculum size than the single culture) resulted in an NH<sub>3</sub> level of 44.5  
262 ppm, which is higher than the sum of the NH<sub>3</sub> level, 23.9 ppm from the individual bacterial  
263 cultures (*S. haemolyticus*: 17.25 ppm; *M. organophilum*: 6.65 ppm). As a result, these  
264 bacteria may have some synergistic effect on NH<sub>3</sub> production, but further studies are needed  
265 to examine this result.

266 In summary, bacteria with a high keratinolytic activity generally favor the production of  
267 NH<sub>3</sub> ( $R = .514$ ;  $P = .017$ ) but are unrelated to the production of VFAs ( $R = .397$ ;  $P = .075$ )  
268 (Figures 6A,B). This system of relations occurs because the production of VFAs is also



269 determined by the amino acid profiles of the protein (branched amino acids) and the lipid and  
270 long-chain fatty acid content such as from skin excretion (gender, age and many other factors  
271 affect the skin excretion)<sup>5</sup> rather than just an overall increase in protein levels via  
272 keratinolysis (James et al<sup>12</sup>). In addition, the type and amount of VFAs production are more  
273 dependent on the bacterial species than in the NH<sub>3</sub> production by deamination, which is a  
274 general process performed by all the bacteria. The ability of the bacteria to proteolyze the  
275 protein and deaminate amino acids to NH<sub>4</sub><sup>+</sup> is a critical step to increase NH<sub>3</sub> emission as  
276 shown by the strong correlation between NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> ( $R = .862$ ;  $P < .001$ ) (Figure 6C).  
277 NH<sub>3</sub> levels are also correlated with VFAs ( $R = .529$ ;  $P = .014$ ) (Figure 6D). This supports the  
278 cooccurrence of the odor but their emission time may vary due to the requirement of different  
279 emission conditions. In general, increasing the loading of skin squames in AC units is likely  
280 to increase the chance of NH<sub>3</sub> and VFA production, but the ultimate VFA production level is  
281 influenced by more variables than NH<sub>3</sub>.

282

### 283 *Effect of temperature on NH<sub>3</sub> production*

284 The NH<sub>3</sub> production by *S. haemolyticus*, *M. organophilum* and *B. thuringiensis* was  
285 significantly affected by temperature (Figure 7A). At 10°C, no NH<sub>3</sub> was detected for all 3  
286 bacteria. When the temperature was increased to 15°C, there was still no detection of NH<sub>3</sub> in  
287 *S. haemolyticus* and *M. organophilum* cultures even though a small amount of NH<sub>3</sub> was

288 measured in *B. thuringiensis* culture (9 ppm). When the temperature was raised higher to  
289 20°C, there was a remarkable increase in NH<sub>3</sub> production by all the bacteria (*S. haemolyticus*:  
290 28 ppm; *M. organophilum*: 11 ppm; *B. thuringiensis*: 30 ppm). A further increase in the  
291 temperature to 30°C did not significantly extend the NH<sub>3</sub> production. The NH<sub>4</sub><sup>+</sup> levels,  
292 keratinolytic activity, and ureases activity of the bacteria also showed a similar response to  
293 the temperature as NH<sub>3</sub> production (Figure 7B-D). These suggest that temperature affects the  
294 bacterial activity. The keratinolytic and ureases activity of the bacteria grew sharply at 20°C,  
295 indicating that 20°C was a critical temperature for the bacteria to begin the odor production in  
296 our experimental setup.

297

#### 298 *Proposed odor control measures in AC units*

299 Cleaning the AC unit using a high pressure spray with coil cleaning agents is the most  
300 common practice at our university to deal with the problem of unpleasant smells. According  
301 to the records, some occupants reported the reoccurrence of unpleasant smells again within a  
302 month after cleaning. This suggests that cleaning the AC units cannot fully resolve the  
303 problem. In some cases, several other methods have been attempted to tackle the odor such as  
304 using activated carbon odor absorbents to absorb the smell in the supply air, cleaning the unit  
305 using ozone, installing UVC light in the unit, and coating photocatalysts on the cooling coil  
306 fin surface. None of these methods worked. Our findings imply that these methods did not

307 work because they cannot stop the skin squama and the odor-causing bacteria entering the  
308 units. Schmidt et al<sup>21</sup> also reported the difficulty in completely inhibiting microbial growth  
309 even when using copper fins instead of aluminum fins in AC units. The 2 *Staphylococcus* spp.  
310 are common skin flora<sup>4</sup> and are frequently found in Hong Kong indoor environments.<sup>22</sup> Thus,  
311 improving the quality of the filter in the AC unit should be an odour control strategy.  
312 Moreover, other interventions that can reduce the amount of skin squames entering the unit  
313 may help alleviate the odor problem.

314 After reporting our study results to the facilities management, they decided to replace the  
315 washable aluminum filter (an average arrestance of 60-66% on ASHRAE 52-76 standard)  
316 (AEROTECH, Hong Kong, China) with a new catalytic activated carbon fiber filter  
317 (ACF-mini V, Osaka Gas, Osaka, Japan) at the return air grille of the units that have frequent  
318 odour complaints as part of a trial study (10 units). The diameter of the fiber ( $\geq 99\%$  carbon  
319 content) is about 10-20  $\mu\text{m}$  with a specific surface area of 700 – 2000  $\text{m}^2/\text{g}$ . This new filter  
320 captures particles more effectively than the old one. Since then, they have not received any  
321 complaints from the occupants for a period exceeding a year. This result suggests the  
322 installation of this new filter is a means of mitigating the odor problem.

323 A laboratory setup (flask conditions) was used in this study to examine the odor  
324 production characteristics of the bacteria. However, in AC units, the environmental condition  
325 is different from the flask condition such as the very high airflow rates in the AC unit

326 compared to the rotary shaking condition in the flask. Moreover, various indoor  
327 environmental conditions could affect the open ACSs system. Ongwandee and Morrison<sup>23</sup>  
328 reported that the presence of acidic (CO<sub>2</sub>) and basic (NH<sub>3</sub>) gases could influence the sorption  
329 of a basic organic pollutant to carpet and latex-painted gypsum board. Similarly, the presence  
330 of these gases may affect the microbial environment and the odour production and emission  
331 process. A wider environmental condition should be considered in the assessment of the  
332 Indoor Air Quality and when deciding the remediation approach.

333

### 334 **Conclusions**

335 This study examined the role of skin squames as a food source that helps some bacteria  
336 colonizing in the AC unit to produce NH<sub>3</sub> and VFAs. It has also identified the odor-causing  
337 bacteria and the corresponding odor production pathway. Several studies have reported  
338 human occupancy as a source of indoor airborne bacteria and the presence of skin flora in the  
339 air. This study demonstrates their potential active role in deteriorating Indoor Air Quality by  
340 colonizing in AC units. Based on the results of this study, our facilities management  
341 determined a strategic odor control measure of replacing the filter, which effectively controls  
342 the odor problem.

343

### 344 **Acknowledgment**

345 This research was funded by the Environment and Conservation Fund (ref: 89/2015)

346

### 347 **Ethics declaration**

348 The university ethical committee approved this study.

349

350

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412



413 **Figure Legends**

414 Figure 1. Ammonia levels of the bacterial culture with skin squames at 30°C. Error bars  
415 represent the standard deviation of replicates (n=3). The change in ammonia levels over the  
416 study period was analyzed by repeated measures one-way ANOVA. Grouping was performed  
417 by post hoc test Duncan analysis. Letters in the parentheses represent different groupings.

418

419 Figure 2. A, ammonium and B, volatile organic acid (VFA) levels in the bacterial cultures  
420 after 3-day incubation at 30°C. Error bars represent the standard deviation of replicates (n=3).  
421 The means between cultures were analyzed by one-way ANOVA. Grouping was performed  
422 by post hoc test Duncan analysis. Letters above the error bars represent different groupings.

423

424 Figure 3. Proposed odor production pathways by the bacteria inhabiting the air-cooling units.

425

426 Figure 4. Keratinolytic activity in the bacterial cultures after 3-day incubation at 30°C. Error  
427 bars represent the standard deviation of replicates (n=3). The means between cultures were  
428 analyzed by one-way ANOVA. Grouping was performed by post hoc test Duncan analysis.  
429 Letters above the bars represent different groupings.

430

431 Figure 5. Urease activity in the bacterial cultures after 3-day incubation at 30°C. Error bars  
432 represent the standard deviation of replicates (n=3). The means between cultures were

433 analyzed by one-way ANOVA. Grouping was performed by post hoc test Duncan analysis.

434 Letters above the bars represent different groupings.

435

436 Figure 6. Pearson's correlation between (A) ammonia levels and keratinolytic activity, (B)

437 volatile organic acids (VFAs) levels and keratinolytic activity, (C) ammonia and ammonium

438 levels, and (D) ammonium and VFAs levels in the bacterial cultures.

439

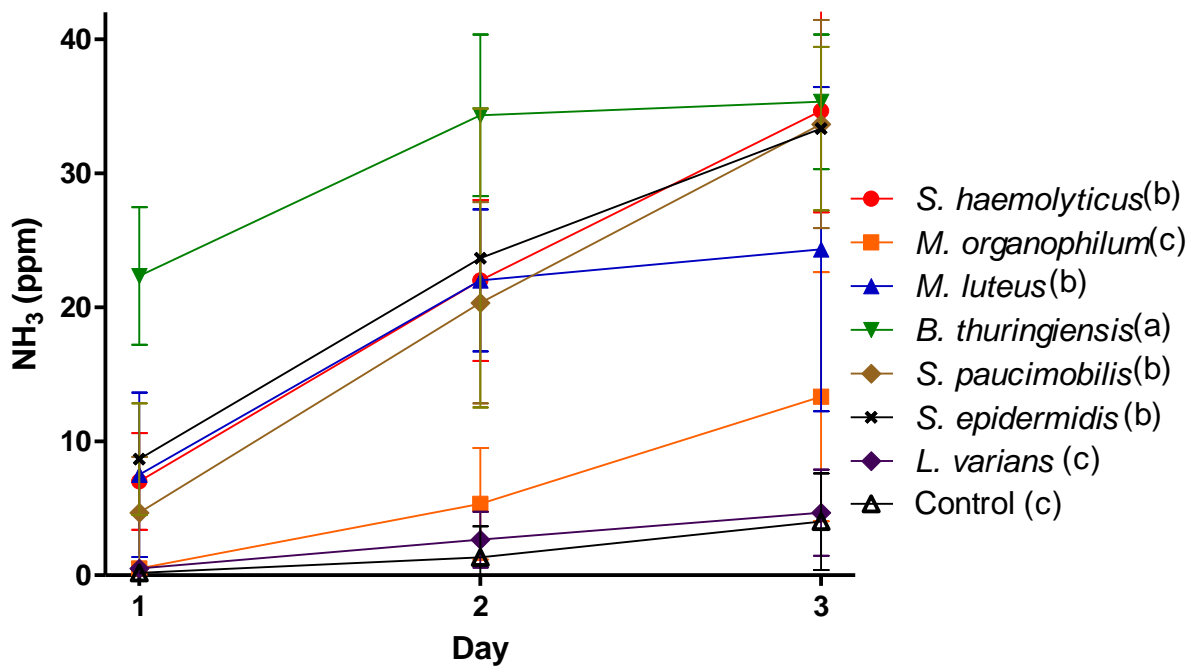
440 Figure 7. Levels of (A) ammonia, (B) ammonium (C) keratinolytic activity, and (D) urease

441 activity measured in the skin squames culture with *Staphylococcus haemolyticus*,

442 *Methylobacterium organophilum*, and *Bacillus thuringiensis* at different temperatures. Error

443 bars represent the standard deviation of replicates (n=3).

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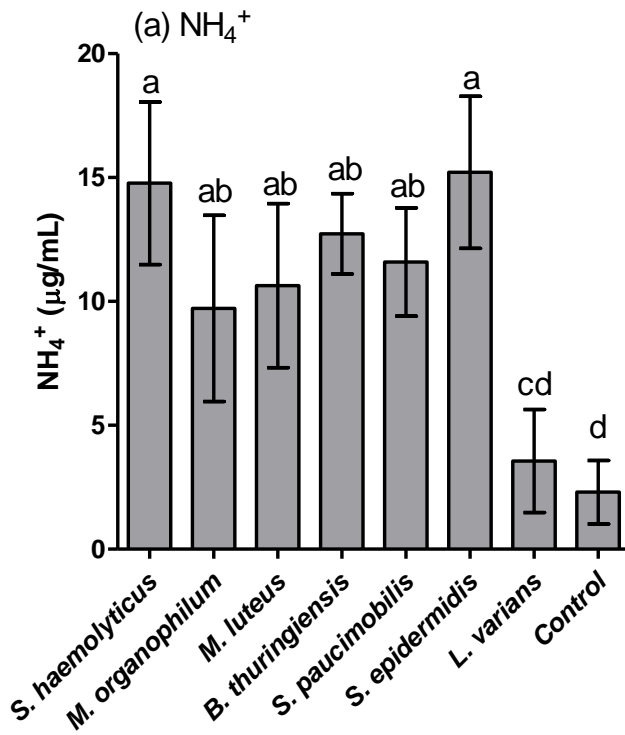


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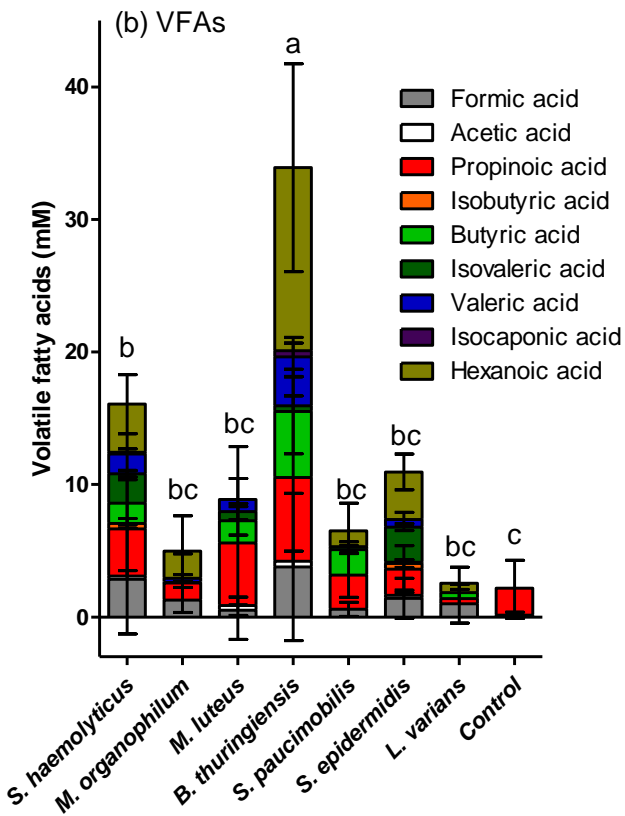
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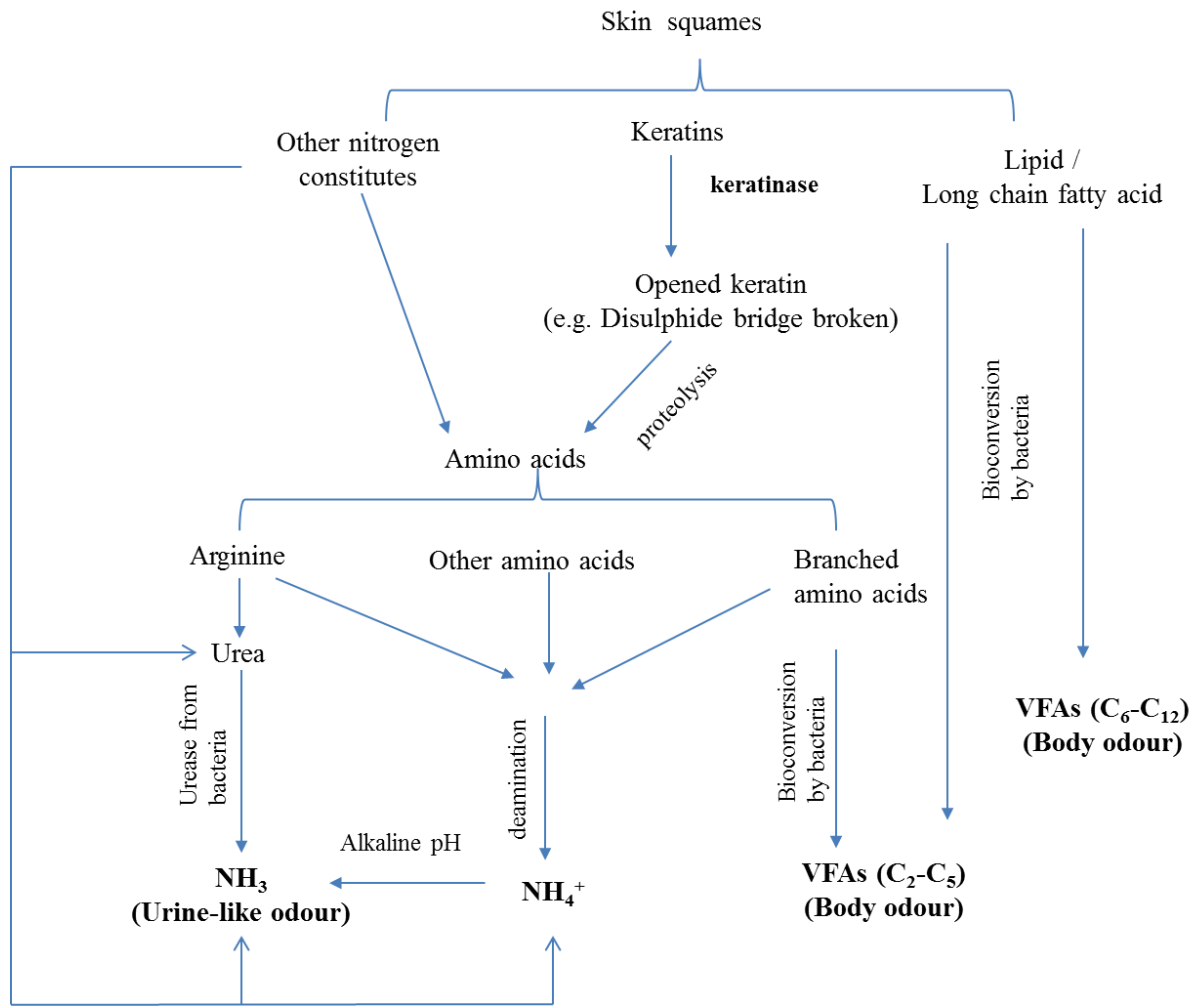
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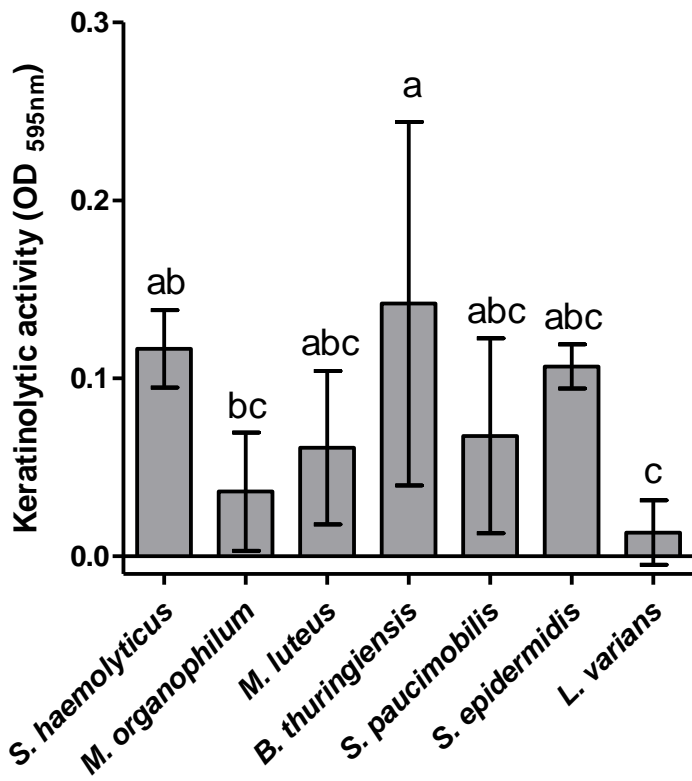
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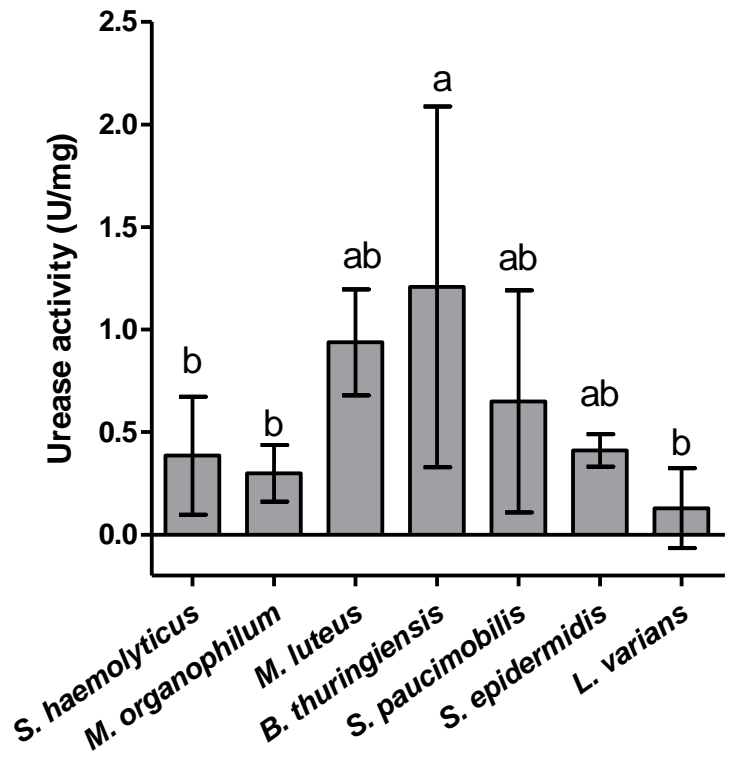
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457 Figure 4.

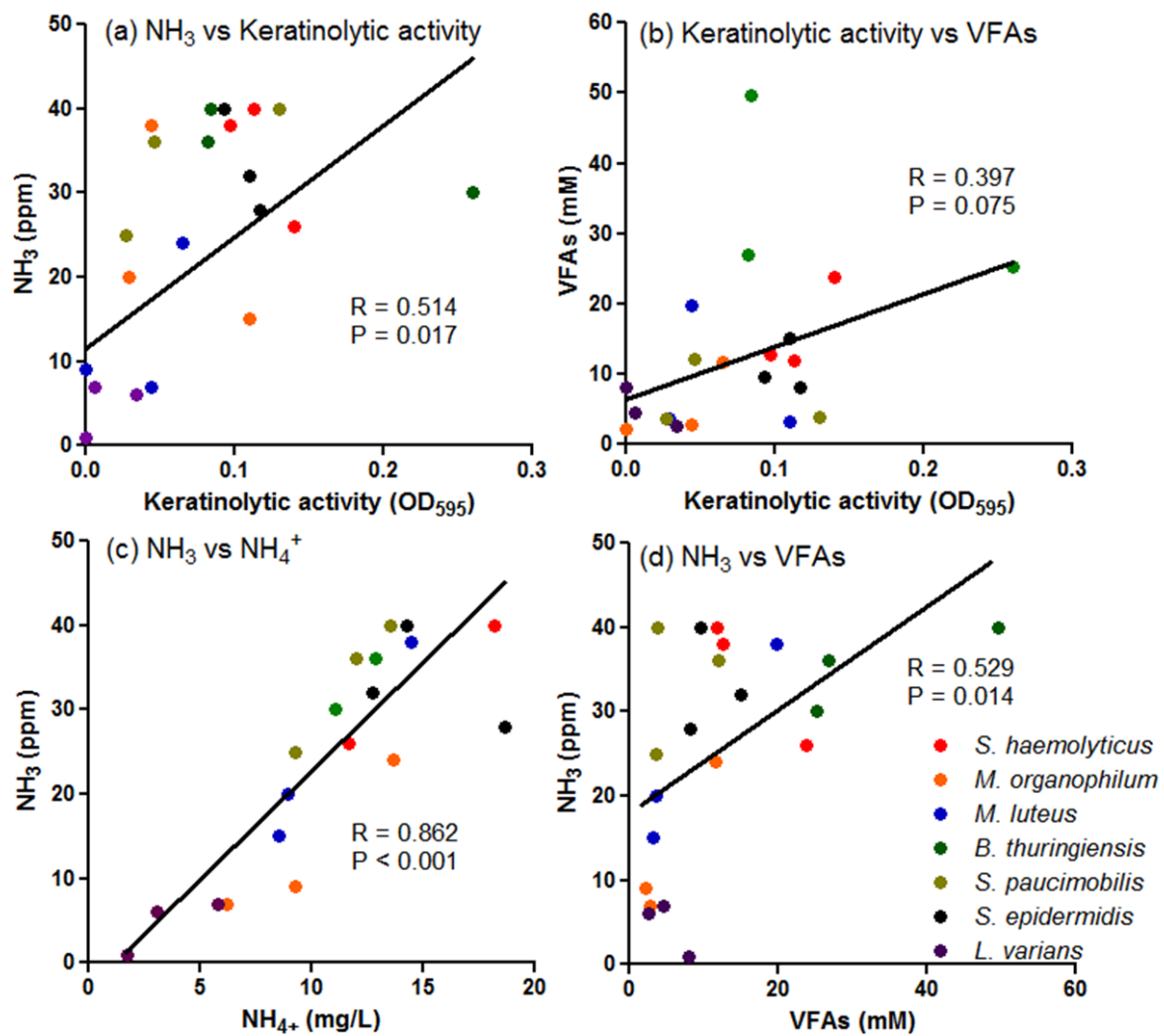
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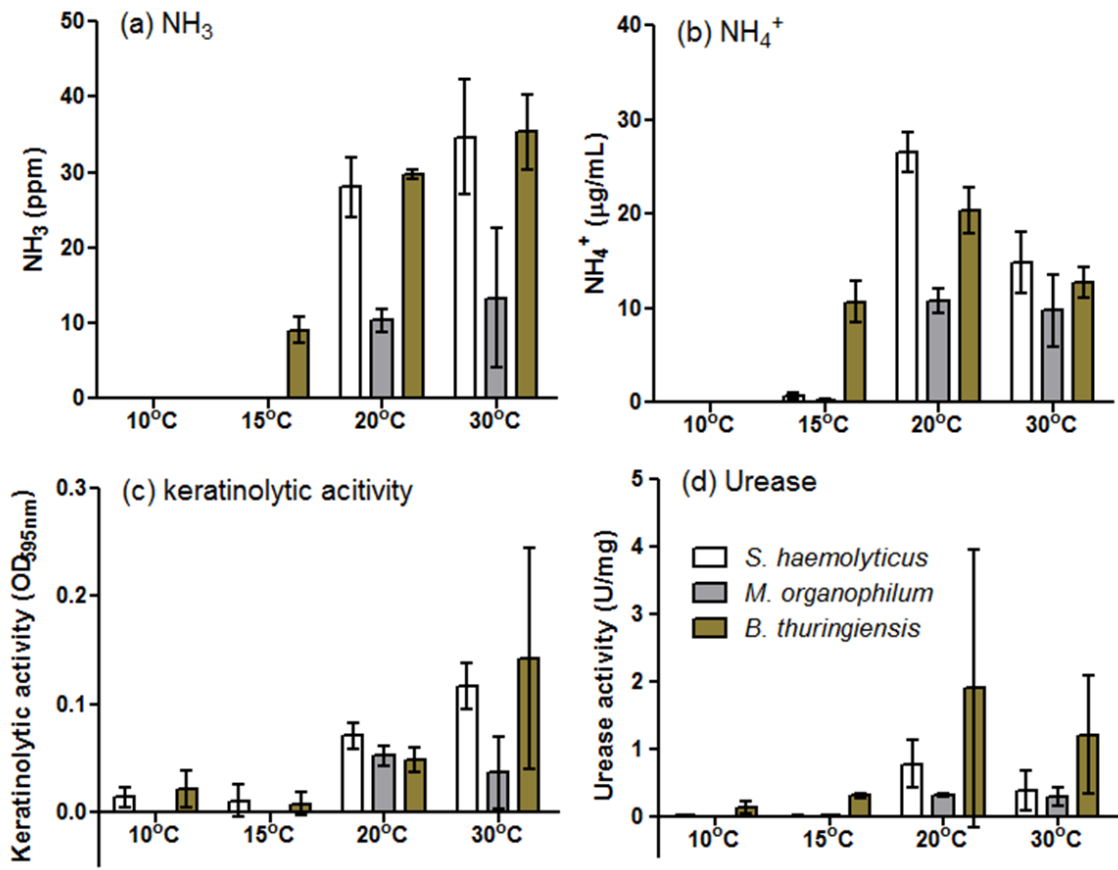
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462 Figure 5



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 464 Figure 6.  
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468 Figure 7.