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

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Abstract

One of the most notable Indoor Air Quality problems is odour emission. This study investigated the potential contribution of skin squames to the production of ammonia (NH_3) and volatile organic acids (VFAs) by 7 bacteria isolated from air-cooling (AC) units with complaints of urine and body odors. Our previous study showed that keratinolytic activity is higher in AC units with odor complaints than those without. In the offices where these units are located, the most likely source of keratins is from human skin squames. Most bacteria can produce NH_3 and VFAs in the skin squame culture. Some correlations between the levels of NH_3, NH_4^+, VFAs, and keratinolytic activity were found. The odour production pathway with skin squames was proposed. *Staphylococcus haemolyticus* was abundant in the AC units with odour problems and had a high level of keratinolytic activity in addition to odour production. For long-term odor control, it is important to reduce the level of skin squames entering the AC units.

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

Practical Implications

This study demonstrates the potential contribution of skin squames to the production of ammonia (NH_3) and volatile organic acids (VFAs) from bacteria colonising in air-cooling (AC) units with complaints of urine and body odors. Understanding the source and mechanism of odour production helps to formulate the control measures. For long-term odour control, it is important to reduce the level of skin squames entering the AC units, such as by improving the filter quality of the air-handling unit. Several studies have reported human occupancy as a source of indoor airborne bacteria and the presence of skin flora in the air. This study demonstrates their potential active role in deteriorating Indoor Air Quality by colonizing in AC units.

Introduction

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

Our previous study found that the level of keratinolytic activity - ability to break down keratins such as through the disulfide bond (-S-S-) to release proteins for further degradation - in AC units is positively correlated to odor complaints.[2-4] This provides a clue to the link between odor production and keratins. The major source of keratins in the indoor environments that we investigated likely comes from the skin squames from occupants. Squames refer to the terminally differentiated, enucleated keratinocytes, which are located at the top layer of the 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.[5] 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.[6] Although the
63 size of skin squames is generally larger than 10 μm,[6] 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 constitutes may
67 serve as nutrients for bacterial growth and odor production in AC units. Acerbi et al.[7] reported
68 an ecological succession of microbial communities in AC units. Hugenholtz et al.[8] and
69 Simmons et al.[9] 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[10] 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.[5,11-12] Propionibacteria,
76 Staphylococci and some other anaerobic bacteria, *Anaerococcus* spp. ferment glycerol and
77 lactic acid to odourous 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.[11] *Staphylococcus* spp. and
80 *Bacillus* spp. are capable of converting branched aliphatic amino acids, leucine to produce a
81 highly odourous iso-valeric acid, a typical foot odourant.[12] Skin microbiology, body odor, and
82 methylotrophic bacteria were reviewed by Kelly and Wood.[5] Not only do the excretes 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.[11] VFAs, such as hexanoic acid, isovaleric acid
86 and isobutyric acid, are widely recognized as being responsible for the unpleasant smells
87 from humans.[5] Ara et al.[12] 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_3), which is a waste
90 product occurring from the breakdown of some amino acids. Ammonium (NH_4^+) 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_3.[2] NH_3 is a pungent gas and has an odor threshold of 5 ppm.
94 High levels of NH_3 (24 ppm) can cause nasal irritation.[13]

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.[1,9] This study aims to test

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

Materials and Methods

Preparation of Skin Squames

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

Bacterial Culture

The bacteria tested were *S. haemolyticus, M. organophilum, Micrococcus luteus, Bacillus thuringiensis, Sphingomonas paucimobilis, Staphylococcus epidermidis,* and *Lysinibacillus varians*.[2] These bacteria were isolated using culture-based methods under aerobic condition in our previous study.[2] 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 30ºC 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.[2] 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 10^8 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 30ºC at 150 rpm for 3
135 days. During the experiment, the levels of NH_3 (detection limit: 0.2 ppm) and VFAs (detection

limit: 1 ppm) in the air were determined daily by Kitagawa precision gas detection tubes (Komyo Rikagaku Kogyo K.K., Kanagawa, Japan). After 3 days, the NH_4^+ concentration, VFAs concentration, urease activity, keratinolytic activity and pH of the bacterial culture were determined as well. The experiments were conducted in triplicates with control setups which had no bacteria added. Since bacteria are not able to grow in the absence of nutrients (e.g. carbon and nitrogen sources), control setups with bacteria and water only are not required. Bacterial growth in the controls was checked using the plate-counting method with tryptic soya agar incubated at 30ºC for 2 days. No bacteria were found on the agar plates.

Temperature effect. The effect of incubation temperature (10, 15 and 20ºC) on NH_3 and VFAs production was examined because the temperature of the cooling coil fin varies during the temperature control cycle. According to our engineers, the general cooling coil temperature is ~ 7ºC. After reaching the set room temperature, the cooling operation turns off automatically, and the cooling coil fin temperature rises with the room temperature immediately until the next cooling cycle. *S. haemolyticus*, *M. organophilum* and *B. thuringiensis* were examined because *S. haemolyticus* and *M. organophilum* were abundant in the AC units with odor complaints,[2] while *B. thuringiensis* showed the highest potential for NH_3 and VFAs production. The experiments were conducted in triplicates.

155 *Analytical Methods*

156 The NH_4^+ concentration and keratinolytic activity were determined by the indophenol
157 blue method[14] and keratinolytic activity assay with keratin azure (Sigma, St. Louis, MO,
158 USA),[15] respectively, as mentioned in our previous study.[2] 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 60ºC 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, isocaponic acid, and hexanoic acid were analyzed in this study.

169

170 *Statistical analysis*

171 The change in the 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 NH_4^+ level, VFAs level, keratinolytic activity and urease activity

between the bacterial cultures on day 3 were compared using one-way ANOVA (SPSS v. 23). Grouping was conducted using post-hoc test Duncan analysis. Differences between means with *P*-values lower than .05 ($P < .05$) were regarded as statistically significant. The correlation between different parameters (NH_3, NH_4^+, keratinolytic activity, urease activity and VFAs) in each culture was analyzed using Pearson's correlation analysis (SPSS v. 23). Correlation with *P*-values lower than .05 ($P < .05$) was regarded as statistically significant.

Results and discussion

Odor production potential of the bacteria

Bacillus thuringiensis showed the fastest rate in the production of NH_3; more than 20 ppm of NH_3 was emitted within a day of incubation (Figure 1). After 3 days, *B. thuringiensis* emitted the highest level of NH_3 (35 ppm), followed by *S. haemolyticus* (34.5 ppm), *S. paucimobilis* (33 ppm), *S. epidermidis* (33 ppm) and *M. luteus* (24 ppm). NH_3 production by *M. organophilum* (13 ppm) and *L. varians* (4.5 ppm) was limited and statistically similar to the control. Although no bacteria were found on the agar plates from the control culture, skin squames may contain NH_4^+ that can convert to NH_3 during incubation. Moreover, slight biological activity from bacteria and enzymes may also contribute to the detectable NH_3 levels in the control setups. NH_4^+ was detected in the bacterial culture after 3 days of incubation, but the levels were largely similar between cultures, except for *L. varians,* which

193 had a similar 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.[11] 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.[5,16] The equilibrium between the chemicals in the air and the solution
205 is dependent on pH. NH_3 as a weak base emits to the air from the conjugate acid, NH_4^+ at
206 high pH levels (acid dissociation starts at pH > 7) because 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

produce both NH_3 and VFAs, our targeted odors with skin squames, but *S. haemolyticus* and *M. organophilum* were not the highest odor producers. In the other words, some factors in the AC unit may favour the population of *S. haemolyticus* and *M. organophilum,* and these factors also facilitate the odor production by *S. haemolyticus.* As a result, *S. haemolyticus,* but not *B. thuringiensis,* was associated with the AC units with odor complaints. *S. haemolyticus* is a common skin flora, which may continuously invade the cooling coil fin environment with the skin squames and take the advantage of consuming the nutrients and, hence, produce the odor. *Methylobacterium spp.* are common bacteria found in AC units due to their general dry-resistance, but their role in producing NH_3 and VFAs is not significant in the single culture condition.[8,9,17,18]

Odor production pathway

The above results demonstrate that most bacteria inhabiting AC units can utilize skin squames to produce NH_3 and VFAs. This part of the study aimed to investigate the pathway of the odor production (Figure 3). Skin squames contain lipids, keratins and other constitutes such as waste products from skin excretion, which can become a source of the odor. Not all bacteria can produce keratinases (a group of extracellular enzymes that performs the keratinolytic activity), and the production of keratinases is only induced by the presence of keratins.[3,4] 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 ($H_2NCHRCOOH$) 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.[19] 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.[3,4] 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).[20] 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).[12] For example, leucine degradation by *Bacillus* spp. produces isovaleric acid and
249 isobutyric acid.[12] 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.[12] 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_3 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_3 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_3. This coculture condition (inoculation with 0.25 mL of 10^8 cfu/mL of each
261 bacterium, half of the inoculum size than the single culture) resulted in an NH_3 level of 44.5
262 ppm, which is higher than the sum of the NH_3 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_3 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_3 (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)[5] rather than just an overall increase in protein levels via
272 keratinolysis (James et al[12]). In addition, the type and amount of VFAs production are more
273 dependent on the bacterial species than in the NH_3 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_4^+ is a critical step to increase NH_3 emission as
276 shown by the strong correlation between NH_3 and NH_4^+ (R = .862; P < .001) (Figure 6C).
277 NH_3 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_3 and VFA production, but the ultimate VFA production level is
281 influenced by more variables than NH_3.
282
283 *Effect of temperature on NH_3 production*
284 The NH_3 production by *S. haemolyticus*, *M. organophilum* and *B. thuringiensis* was
285 significantly affected by temperature (Figure 7A). At 10°C, no NH_3 was detected for all 3
286 bacteria. When the temperature was increased to 15°C, there was still no detection of NH_3 in
287 *S. haemolyticus* and *M. organophilum* cultures even though a small amount of NH_3 was

288 measured in *B. thuringiensis* culture (9 ppm). When the temperature was raised higher to
289 20ºC, there was a remarkable increase in NH_3 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 30ºC did not significantly extend the NH_3 production. The NH_4^+ levels,
292 keratinolytic activity, and ureases activity of the bacteria also showed a similar response to
293 the temperature as NH_3 production (Figure 7B-D). These suggest that temperature affects the
294 bacterial activity. The keratinolytic and ureases activity of the bacteria grew sharply at 20ºC,
295 indicating that 20ºC 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[21] 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[4] and are frequently found in Hong Kong indoor environments.[22] 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 (≥99% carbon
319 content) is about 10-20 μm with a specific surface area of 700 – 2000 m^2/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

compared to the rotary shaking condition in the flask. Moreover, various indoor environmental conditions could affect the open ACSs system. Ongwandee and Morrison[23] reported that the presence of acidic (CO_2) and basic (NH_3) gases could influence the sorption of a basic organic pollutant to carpet and latex-painted gypsum board. Similarly, the presence of these gases may affect the microbial environment and the odour production and emission process. A wider environmental condition should be considered in the assessment of the Indoor Air Quality and when deciding the remediation approach.

Conclusions

This study examined the role of skin squames as a food source that helps some bacteria colonizing in the AC unit to produce NH_3 and VFAs. It has also identified the odor-causing bacteria and the corresponding odor production pathway. Several studies have reported human occupancy as a source of indoor airborne bacteria and the presence of skin flora in the air. This study demonstrates their potential active role in deteriorating Indoor Air Quality by colonizing in AC units. Based on the results of this study, our facilities management determined a strategic odor control measure of replacing the filter, which effectively controls the odor problem.

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346

347 **Ethics declaration**

348 The university ethical committee approved this study.

349

350

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412

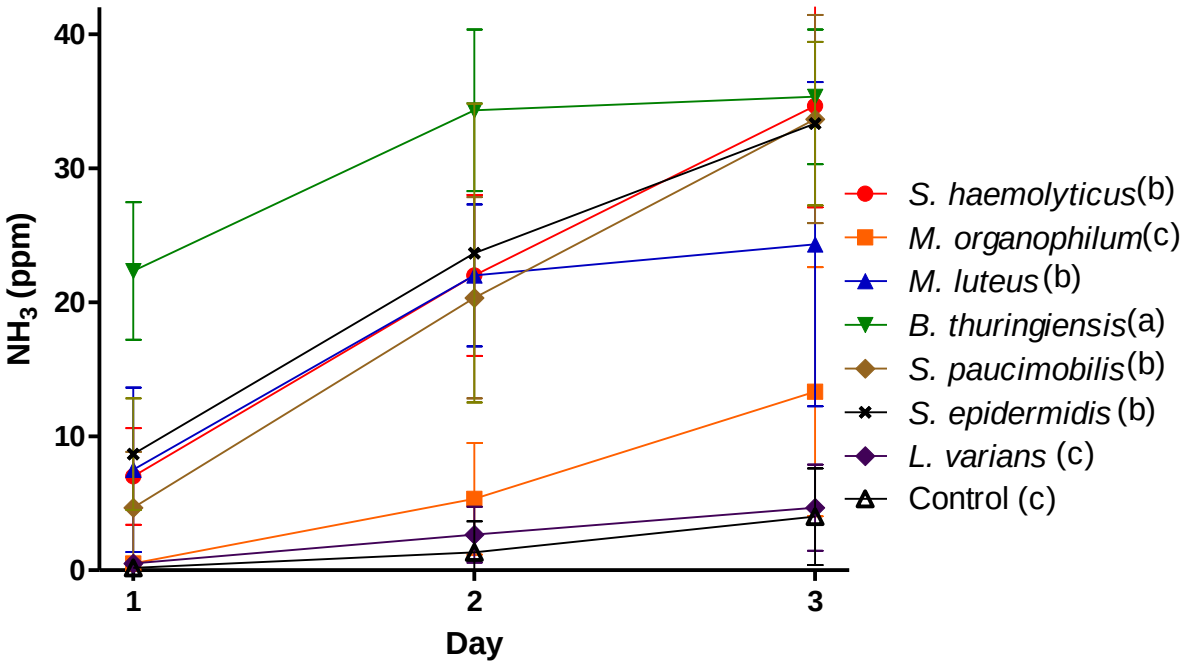
413 **Figure Legends**

414 Figure 1. Ammonia levels of the bacterial culture with skin squames at 30ºC. 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 30ºC. 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 30ºC. 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 30ºC. Error bars
432 represent the standard deviation of replicates (n=3). The means between cultures were

analyzed by one-way ANOVA. Grouping was performed by post hoc test Duncan analysis. Letters above the bars represent different groupings.

Figure 6. Pearson's correlation between (A) ammonia levels and keratinolytic activity, (B) volatile organic acids (VFAs) levels and keratinolytic activity, (C) ammonia and ammonium levels, and (D) ammonium and VFAs levels in the bacterial cultures.

Figure 7. Levels of (A) ammonia, (B) ammonium (C) keratinolytic activity, and (D) urease activity measured in the skin squames culture with *Staphylococcus haemolyticus*, *Methylobacterium organophilum,* and *Bacillus thuringiensis* at different temperatures. Error bars represent the standard deviation of replicates (n=3).

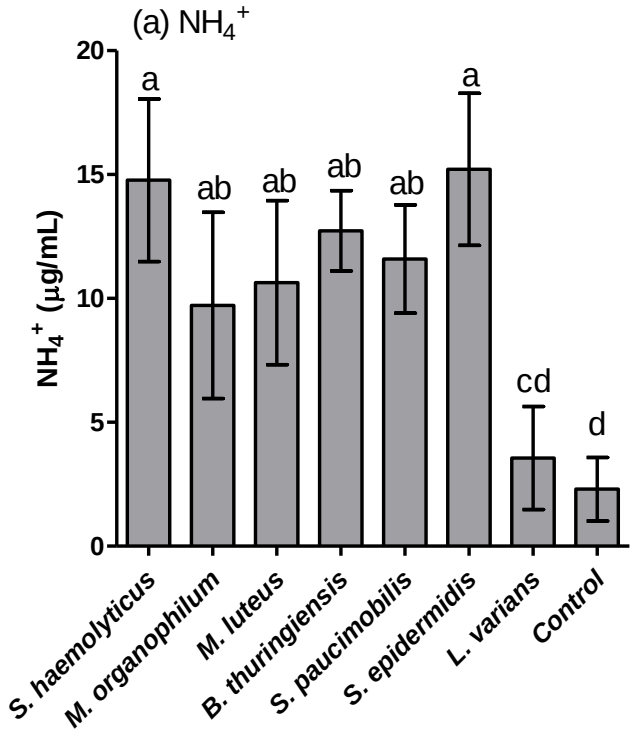


445

446

447 Figure 1.

448



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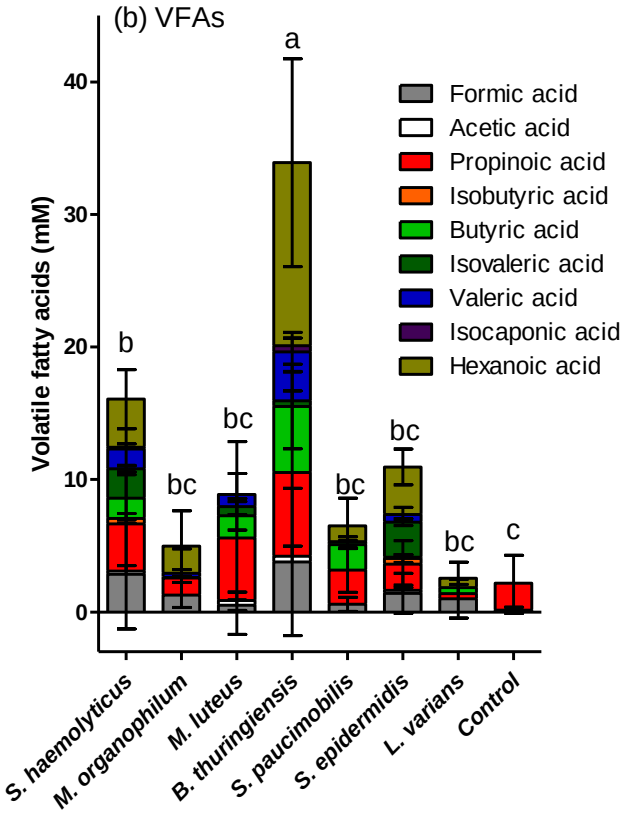


450

451 Figure 2.

452

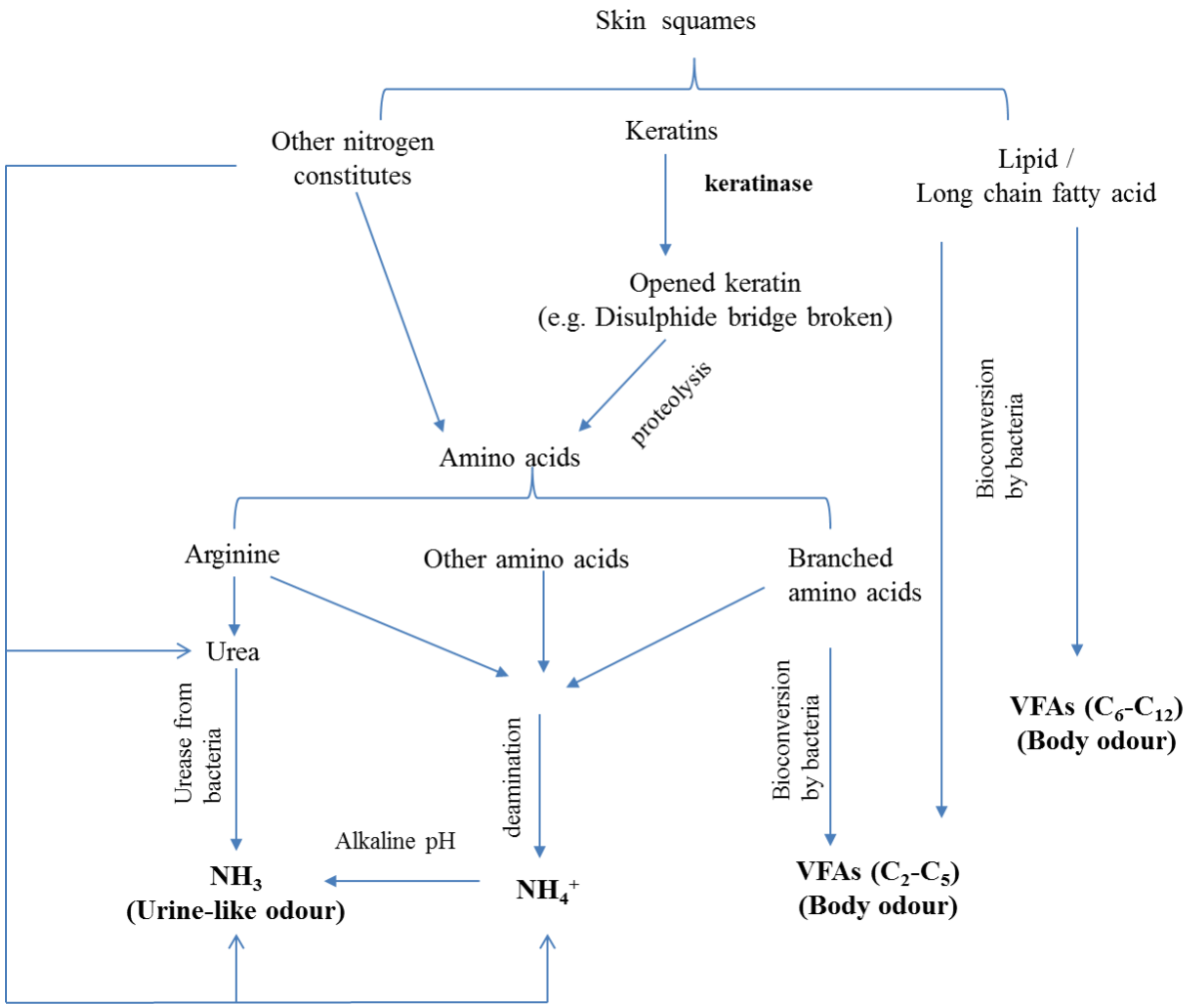
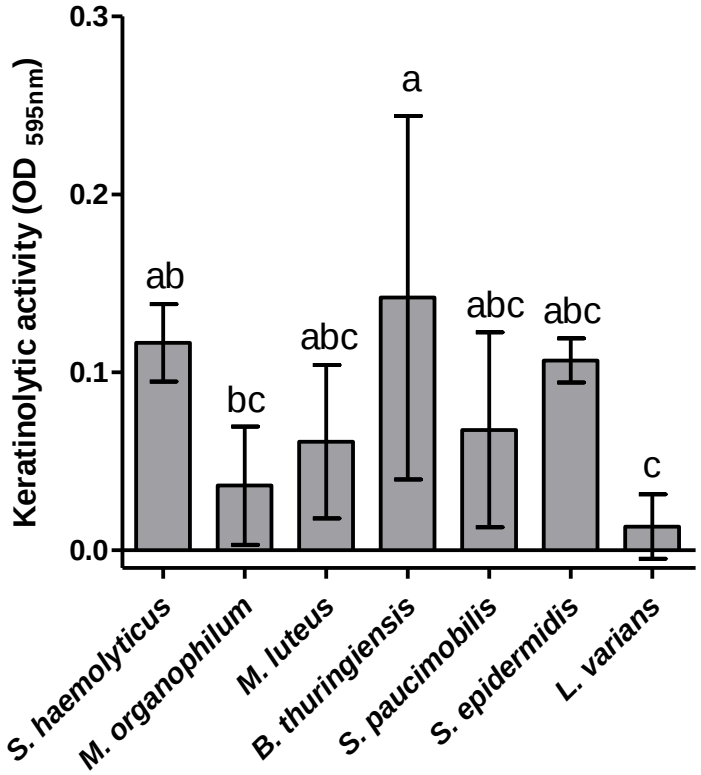

Skin squames
Other nitrogen constitutes
Keratins
keratinase
Lipid / Long chain fatty acid
Opened keratin (e.g. Disulphide bridge broken)
proteolysis
Bioconversion by bacteria
Amino acids
Arginine
Other amino acids
Branched amino acids
Urea
Urease from bacteria
deamination
Bioconversion by bacteria
VFAs (C_6-C_{12}) (Body odour)
Alkaline pH
NH_3 (Urine-like odour)
NH_4^+
VFAs (C_2-C_5) (Body odour)

Figure 3.



456

457 Figure 4.

458

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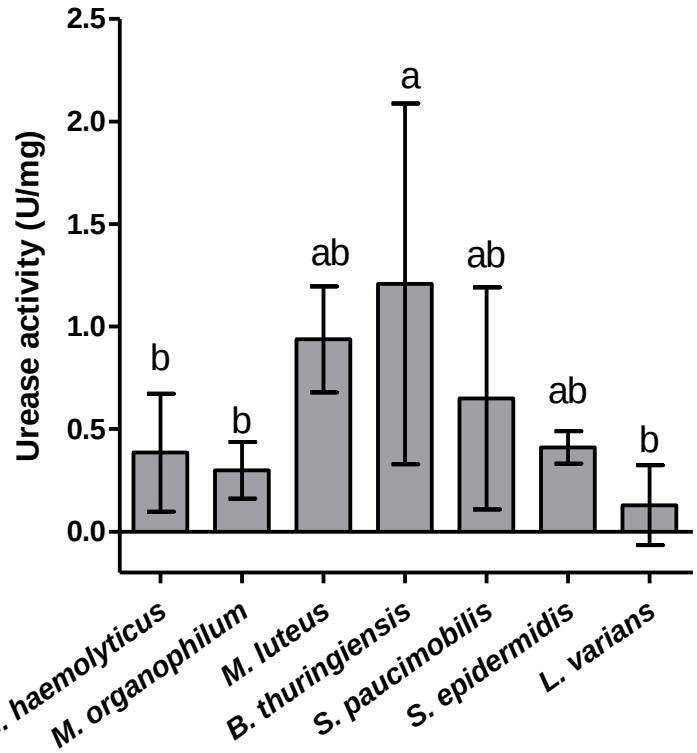

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462 Figure 5

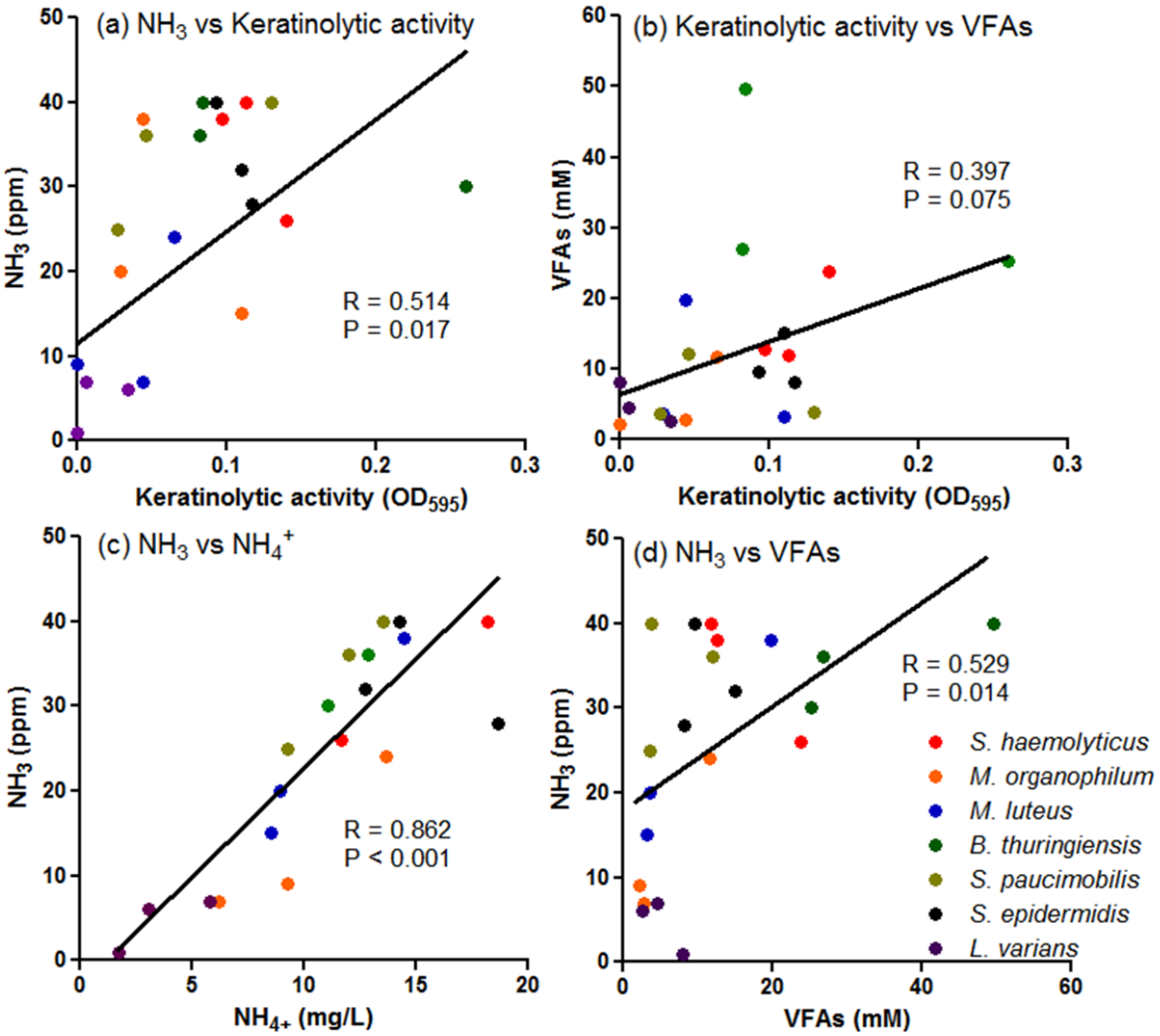
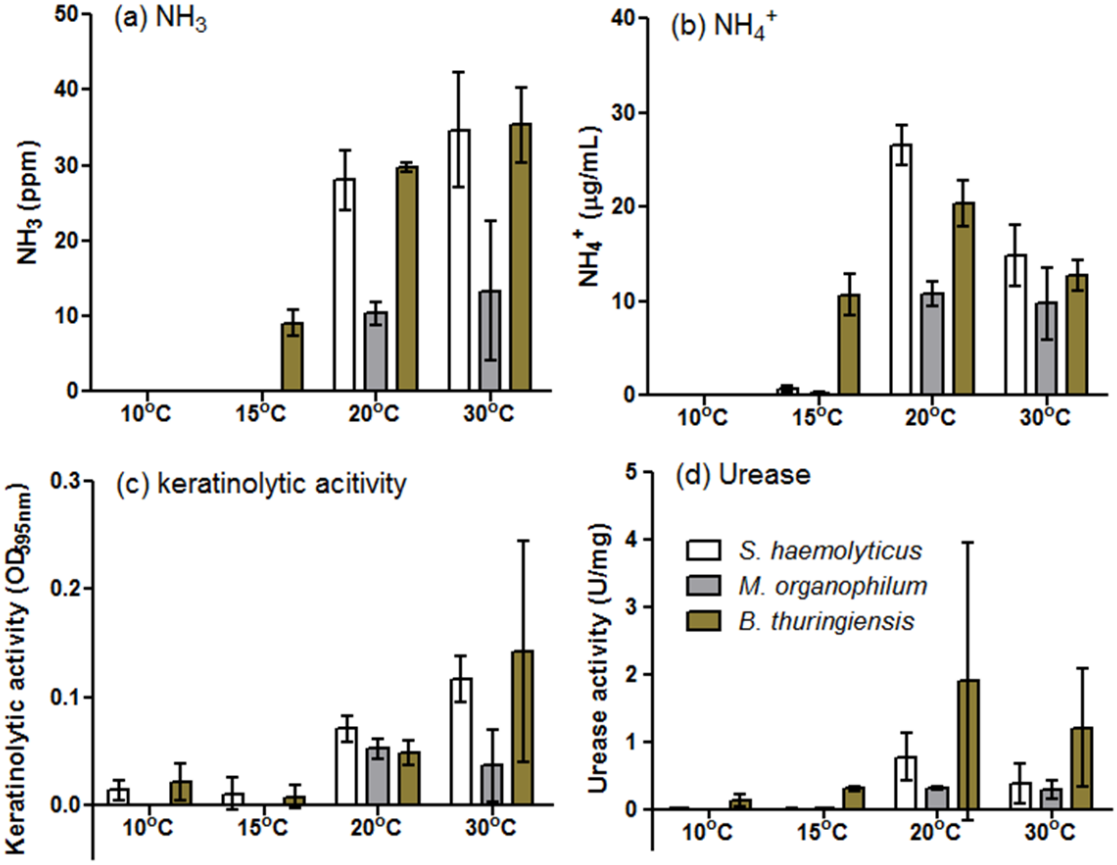

(a) NH3 vs Keratinolytic activity
NH3 (ppm)
Keratinolytic activity (OD595)
R = 0.514
P = 0.017
(b) Keratinolytic activity vs VFAs
VFAs (mM)
Keratinolytic activity (OD595)
R = 0.397
P = 0.075
(c) NH3 vs NH4+
NH3 (ppm)
NH4+ (mg/L)
R = 0.862
P < 0.001
(d) NH3 vs VFAs
NH3 (ppm)
VFAs (mM)
R = 0.529
P = 0.014
S. haemolyticus
M. organophilum
M. luteus
B. thuringiensis
S. paucimobilis
S. epidermidis
L. varians

Figure 6.



467

468 Figure 7.