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Exposure Assessment of Airborne Bacteria Emitted from Swine Manure Composting Plant

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Abstract: This study was performed to investigate the distribution characteristics of airborne bacteria emitted from swine manure composting plants. The types of swine manure composting plants selected for the survey in this study were as follows: screw type, rotary type, and natural dry type. Mean levels of airborne bacteria in swine manure composting plants were 7428 (±1024) CFU m^{-3} for the screw type, 3246 (±1407) CFU m^{-3} for the rotary type, and 5232 (±1217) CFU m^{-3} for the natural dry type, respectively. Based on the results obtained from this study, the swine manure composting plant operated by screw type showed the highest concentration of airborne bacteria, followed by the natural dry type and rotary type. The monthly concentration of airborne bacteria was the highest in August and the lowest in November, regardless of the type of swine manure composting plant. The respirable size of airborne bacteria accounted for about 50% of the total. The ratio of respirable to the total quantity of airborne bacteria was 50%. The correlation relationships between airborne bacteria and environmental factors (temperature, relative humidity, particulate matters, and odor) were not found to be significant in the swine manure composting plants. The predominant genera of airborne bacteria identified were *Micrococcus* spp., *Staphylococcus* spp., *Escherichia (E-coli)* spp., *Enterococcus* spp., *and Enterobacteriaceae* spp.

Keywords: airborne bacteria; swine manure; composting plant; screw; rotary; impactor

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1. Introduction

Currently, plants that treat organic wastes such as swine manure have expanded in South Korea because the Korea government promotes "low carbon green growth" as a new national future vision. Within these facilities, however, organic waste decomposes at high temperatures, generating a large amount of air pollutants, with a very high concentration. In particular, it was reported that bioaerosols such as airborne bacteria are discharged in relatively high quantities [1].

Biohazard factors, i.e., bioaerosols, can cause infectious diseases or allergic respiratory diseases, such as asthma, rhinitis, and bronchitis, when introduced into the body through respiration [2,3]. Therefore, the increase in the amount of such bioaerosols has the potential for simultaneously increasing the incidence of environmental diseases. In addition, because the adverse effects of bioaerosols are one of the factors that threaten, not only human health, but also livestock productivity, it acts as an inhibitor of the production of safe livestock products, as well as possibly causing a lack of guaranteed safe diets for humans.

Since organic wastes such as livestock manure, sewage sludge, and food waste are raw materials that play a role as a growth and nutrient source for microorganisms, compost creates good environmental conditions in which many types of microorganisms can survive [4]. In the fermentation stage, where air is introduced into the compost pile during the composting period, most of the microorganisms are killed, because aerobic decomposition of organic matter is rapidly induced and the compost pile maintains a high temperature condition, of 60 °C or higher. However, some thermophilic bacteria, such as Thermoactinomyces sp., survive and are known to cause serious respiratory disease when exposed to humans by being released into the air when turning the compost pile [5]. Since air propagation is the main route for the exposure of bioaerosols to residents near the organic waste composting plant, studies on the movement route due to atmospheric diffusion have already been conducted overseas [6–8].

However, there is little domestic information about exposure levels of bioaerosols derived from swine manure composting plants, so a basic environmental impact assessment is required. Additionally, data on bioaerosols emitted from swine manure composting plants specifically in South Korea should be established, given that the composition of swine manure and composting operation modes in South Korea are different from those in other countries. Thus, an objective of this study was to investigate the distribution characteristics of airborne bacteria emitted from swine manure composting plants in South Korea and to utilize the data for protecting the health of workers and neighbors from exposure to airborne bacteria.

2. Materials and Methods

2.1. Subject

Three types of swine manure composting plants situated in Jeju city, South Korea were selected based on the fermentation mode of the compost pile: screw type, rotary type, and natural dry type. Table 1 summarizes the main characteristics of the swine manure composting plants investigated in this study.

Table 1. Profile of swine manure composting plants investigated in this study.

Site	Reactor Type	Turning Mode	Treatment Capacity	Location
1	Cross	Screw	10 (7.5) * ton/day	
2	Cross	Rotary	5 (1) ton/day	Jeju
3	Pile	Natural dry	3 (1.5) ton/day	

* Practical treatment capacity.

2.2. Measurement

During the period from May 2019 to April 2020, three composting plants were visited once every month. Air samples for measuring airborne bacteria were taken l m above the center of the swine manure composting plant between 13:00 p.m. and 17:00 p.m. For each measurement, a sample was collected, repeating 3 times, and the average was taken as a representative value.

A six-stage viable particulate cascade impactor (Model 10-800, Andersen Inc, Cleves, OH, USA) set to the flow amount of 28.3 L/min, which had been previously utilized by Kim et al. [9], was used for sampling airborne bacteria, and the aerodynamic diameter range for each stage were as followed: stage 1 (>7.0 μm), stage 2 (4.7–7.0 μm), stage 3 (3.3–4.7 μm), stage 4 (2.1–3.3 μm), stage 5 (1.1–2.1 μm), and stage 6 (0.65–1.1 μm). Air sampling was carried out for 8 to 12 min, according to the environmental situation of the measurement locations. Before sampling, the inside of the sampler was disinfected with 70% alcohol and then was inserted with the agar plate according to the collection stage. Trypticase soy agar (Lot 2087730, Becton Dickinson and Company, Franklin, NJ, USA), where cycloheximide 500 mg was added to suppress the growth of fungi, was used as bacterial culture medium. The culture media for which sample collection were finished were immediately taken to the microbe laboratory and were cultured in the incubator for 1–2 days under a 37 °C condition. The concentration of airborne bacteria, i.e., cfu/m^3, was calculated by dividing by the air volume (m^3) the value obtained from counting the colonies formed on the culture medium after the process of culturing (Equations (1) and (2)). The concentration value was finally presented through correction by the application of the "positive hole correction" method.

$$\text{CFU (Colony Forming Unit)/m}^3 = \text{Colony counted on agar plate/Air volume (m}^3\text{)} \quad (1)$$

$$\text{Air volume } (m^3) = 28.3 \text{ L/min} \times \text{sampling time (min)}/10^3 \quad (2)$$

The genera of all the cultured airborne bacteria were identified according to the classification method from Bergey's manual and, after dying the bacteria using Gram's method, additional identification was carried out by conducting biochemical tests with an automated microbial identification system, VITEK (Model VITEK 32 system, bioMerieux Inc., Durham, France).

In order to verify the statistical correlation with airborne bacteria, the environmental factors of the swine manure composting plants simultaneously measured at the site were temperature, relative humidity, particulate matters (TSP, PM_{10}, $PM_{2.5}$ and PM_1), and odor. They were monitored directly using a digital thermohygrometer (608-H1, Testco, Sunnyvale, Germany) for temperature and humidity, portable dust monitor (Dustmate, Turnkey Instruments Ltd., Cheshire, UK) for particulate matters, and hand-held odor monitor (OMX-SR, Shinyei, Japan) for odor.

2.3. Data Analysis

The Statistical Analysis System (SAS) package (SAS/Stat 9.1, SAS Institute Inc., Cary, NC, USA) was used for data analysis of the measured field data. First, through the Shapiro–Wilk test, it was found that the measured data had a normal distribution and the measured values were presented as arithmetic mean and standard deviation. Analysis of Variance ANOVA and Duncan's multiple comparison analysis methods were applied to compare the concentration difference of the internal airborne bacteria, according to the type of swine manure composting plant and the emission amount of airborne bacteria generated at each turning time of the compost pile. The correlation between airborne bacteria and environmental factors in the swine manure composting plants was verified for statistical significance by applying Pearson's correlation test method.

3. Results

3.1. Monthly Concentration Distribution of Airborne Bacteria According to the Type of Swine Manure Composting Plant

The Figure 1 shows the monthly concentration trend of airborne bacteria from swine manure composting plants operated with the three types of compost pile turning modes. In case of the screw type, the mean concentration of airborne bacteria was 7728 (±1024) CFU m^{-3} and its maximum and minimum levels were 12,261 (±1449) CFU m^{-3} in August and 1737 (±660) CFU m^{-3} in November, respectively. In the case of the rotary type, the mean concentration of airborne bacteria was 3246 (±1407) CFU m^{-3} and its maximum and minimum levels were 8622 (±2399) CFU m^{-3} in August and 1195 (±385) CFU m^{-3} in November, respectively. In the case of the dry type, the mean concentration of airborne bacteria was 5232 (±1217) CFU m^{-3} and its maximum and minimum levels were 12,756 (±2168) CFU m^{-3} in August and 2142 (±437) CFU m^{-3} in November, respectively. Based on the results obtained from this study, the mean concentration of airborne bacteria was highest in the swine manure composting plant operated with the screw type, followed by the dry type and rotary type ($p < 0.05$). Regardless of the compost pile turning mode, the monthly level of airborne bacteria in the swine manure composting plants was highest in August and lowest in November. Additionally, all three composting plants exceeded the domestic indoor standard guideline (800 CFU m^{-3}) of airborne bacteria.

3.2. Comparison of Airborne Bacteria Emitted from Swine Manure Composting Plants According to Agitation Time of Compost Pile

The Figure 2 shows the concentration pattern of airborne bacteria according to the agitation time (before, during, and after turning) of the swine manure compost pile. The turning period was one hour, and, after turning, air samples were taken for one hour. The mean values of airborne bacteria were 1537 (±125) CFU m^{-3} before turning, 1413 (±899) CFU m^{-3} during turning, and 1131 (±150) CFU m^{-3} after turning, respectively. As a result of these measurements, it was analyzed that the difference in the concentration of airborne bacteria according to agitation time of the compost pile was not statistically significant ($p > 0.05$).

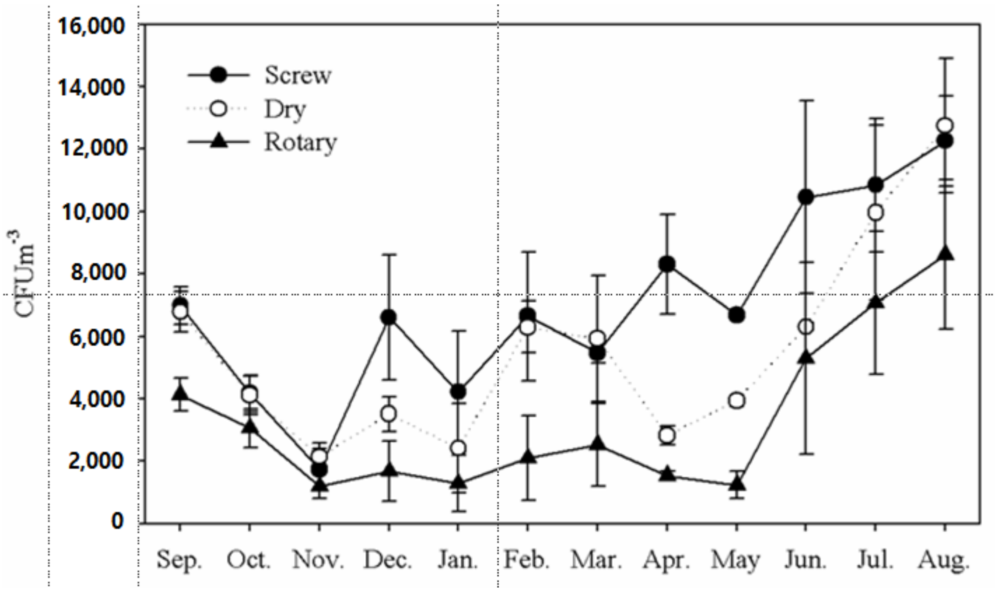

Figure 1. Monthly mean concentration trend of airborne bacteria in swine manure composting plants.

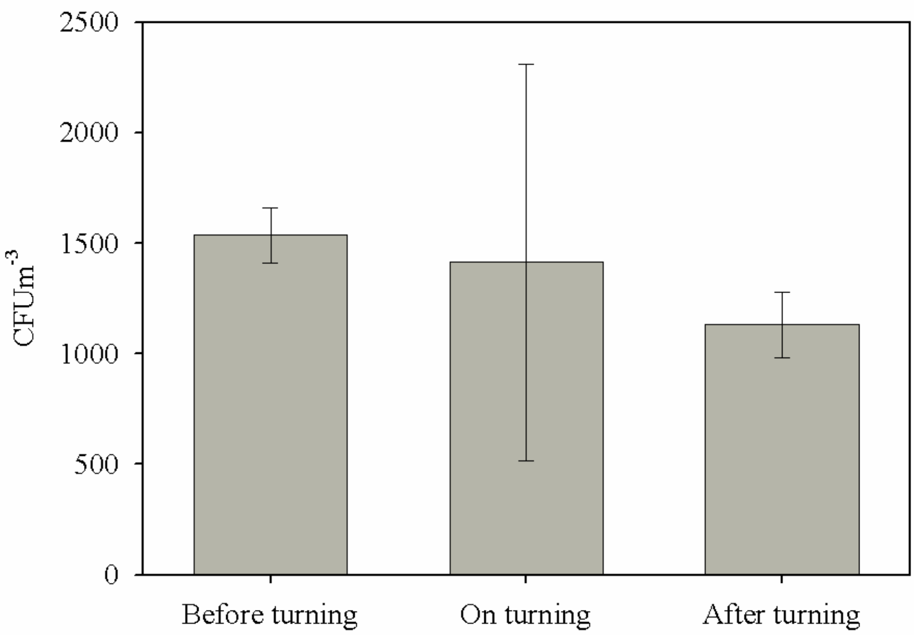

Figure 2. Mean level of airborne bacteria in swine manure composting plants according to the agitation time of the compost pile.

3.3. *Size Distribution Characteristics of Airborne Bacteria According to the Type of Swine Manure Composting Plant*

As presented in the Figure 3, the size distribution characteristics of airborne bacteria in the swine manure composting plants were as follows: 56% (screw), 36% (rotary), and 38% (dry) in stage 1 (>7.0 μm); 2% (screw), 7% (rotary), and 19% (dry) in stage 2 (4.7–7.0 μm); 8% (screw), 7% (rotary), and 3% (dry) in stage 3 (3.3–4.7 μm); 7% (screw), 7% (rotary), and 7% (dry) in stage 4 (2.1–3.3 μm); 8% (screw), 36% (rotary), and 12% (dry) in stage 5 (1.1–2.1 μm); 19% (screw), 8% (rotary), and 21% (dry) in stage 6 (0.65–1.1 μm). Stage 1 (>7.0 μm) showed the highest frequency rate among the particle size ranges, regardless of the type of swine manure composting plant. In the six-stage viable particulate cascade impactor, the proportions of airborne bacteria 0.65 µm or more and 4.7 µm or less (stage 3–6), that is within the respiratory particle size range, of the total concentration were 42% for the screw mode, 57% for the rotary mode, and 43% for dry mode, respectively.

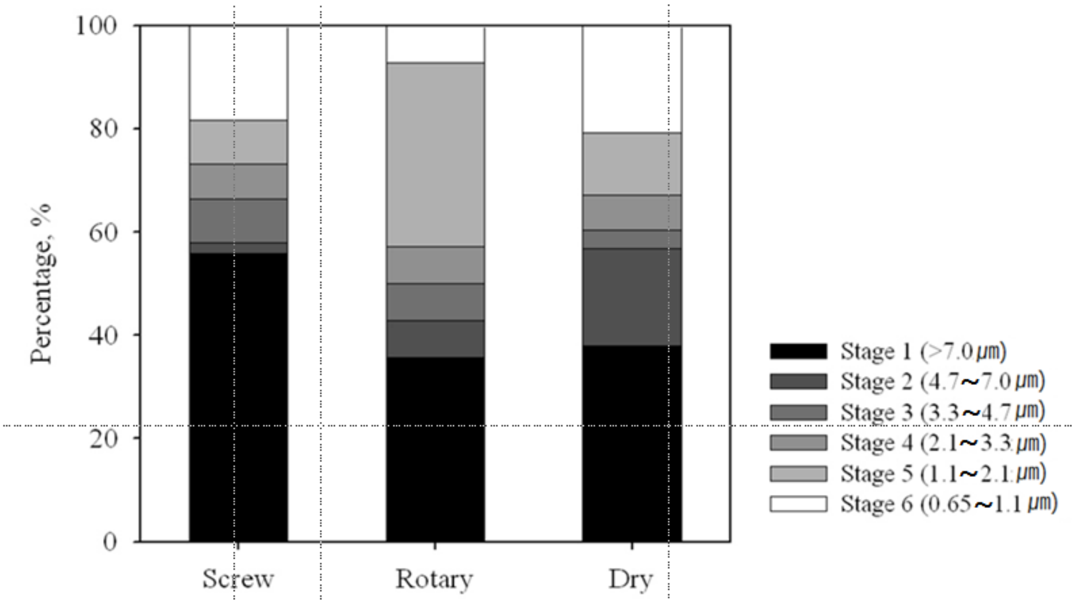

Figure 3. Size distribution characteristics of airborne bacteria in swine manure composting plants.

3.4. Assoiciation between Airborne Bacteria and Environmental Factors in Swine Manure Composting Plants

Table 2 represents the statistical correlation relationships between airborne bacteria and environmental factors investigated in the swine manure composting plants. There were no significant correlation relationships among them, except for temperature/$PM_{2.5}$ (r = −0.776), R.H./PM_{10}(r = 0.752), and $PM_{2.5}$/PM_1(r = 0.722).

Table 2. Correlation relationships between airborne bacteria and indoor environmental factors.

	Airborne Bacteria	Temp.	RH	TSP	PM_{10}	$PM_{2.5}$	PM_1	Odor
Airborne bacteria								
Temp.	0.219							
RH	−0.157	−0.367						
TSP	0.045	0.078	0.470 *					
PM_{10}	0.201	−0.266	0.615 **	0.752 **				
$PM_{2.5}$	−0.008	−0.776 **	0.383	−0.303	0.150			
PM_1	0.060	−0.419	0.127	−0.302	−0.178	0.722 **		
Odor	−0.132	0.150	0.043	−0.017	0.009	0.069	0.144	

* $p < 0.05$, ** $p < 0.01$.

3.5. Qualitative Analysis of Airborne Bacteria, According to the Type of Swine Manure Composting Plant

Table 3 presents the identification results of airborne bacteria from swine manure composting plants, according to fermentation mode. The airborne bacterial species with a detection rate of 10% or more compared to the total were *Micrococcus* spp. (23.3%), *Staphylococcus* spp. (19.3%), *Escherichia* spp. (10.6%), *Enterococcus* spp. (11.9%), and *Enterobacteriaceae* spp. (10.8%) for the screw type; *Micrococcus* spp. (24.1%), *Enterococcus* spp. (14.3%), *Escherichia* spp. (13.4%), *Staphylococcus* spp. (12.8%), and *Enterococcus* spp. (11.3%)

for the rotary type; and *Escherichia* spp. (20.1%), *Micrococcus* spp. (18.9%), *Enterococcus* spp. (13.5%), *Staphylococcus* spp. (11.7%), and *Enterobacteriaceae* spp. (10.2%) for the dry type.

Table 3. Identification of Airborne Bacteria in Swine Manure Composting Plants (Unit: %).

	Screw Type	Rotary Type	Dry Type
Aeromonas spp.	0.2 *	0.4	0.6
Bacillus spp.	5.5	2.8	5.3
Corynebacterium spp.	4.1	4.4	4.2
Enterobacteriaceae spp.	10.8	10.2	10.2
Enterococcus spp.	11.9	14.3	13.5
Escherichia(E-coli) spp.	12.6	15.4	22.1
Micrococcus spp.	23.3	24.1	18.9
Nocardia spp.	3.8	4.4	3.1
Pseudomonas spp.	1.8	1.5	3.8
Staphylococcus spp.	19.3	12.8	11.7
Streptococcus spp.	2.5	3.4	2.3
Unknown	4.2	4.3	4.3
Total	100.0	100.0	100.0

* Detection rate.

4. Discussion

As a result of the field investigation, the monthly concentration distribution of airborne bacteria in the swine manure composting plants did not show a constant change, regardless of fermentation mode. In general, it was found that the concentration of airborne bacteria increased during the spring/summer season when the temperature was high, but it is estimated that the variation in the environmental conditions (temperature, humidity, airflow, etc.) in the swine manure composting plants at the time of measurement had a greater influence on the concentration of airborne bacteria. This assumption is supported by the relatively decreased concentration of airborne bacteria measured in May, when the outdoor temperature is mild.

In Korea, there have not been reports surveying the amount of airborne bacteria in a facility that composts organic waste, such as animal manure. Considering the results of previous research, it was reported as follows: 3204 CFU m^{-3} [6], 10^3~10^5 CFU m^{-3} [10], and 261~18,700 CFU m^{-3} [1]. Fischer et al. [11] found that during the process of stirring the compost pile, thermophilic bacteria were discharged into the air, up to a maximum of 2.4×10^6 CFU m^{-3}. According to the most recently published research results, it was reported that mesophilic and cryogenic airborne bacteria were generated at an average level of 308 CFU m^{-3} and 328 CFU m^{-3}, respectively [12]. Reviewing the results of previous studies, it was confirmed that there is a significant difference in the concentration of airborne bacteria among research works, and there are also a significant differences in comparison with the results of this study. This would be mainly caused by the differences in composting raw materials with different properties. In this study, composting plants based on swine manure were the subject of the investigation, whereas in the case of previous studies, the plants composted general waste, such as municipal solid waste and food waste. In addition, the fact that meteorological factors such as temperature, humidity, wind speed, and season, which affect the generation of airborne bacteria during the composting process, were different from each other at the time of measurement may be one of the reasons for variance [13–15].

Compared with 800 CFU m^{-3}, which is the standard value of airborne bacteria in Korea, it was shown that all measured monthly concentrations exceeded this, regardless of the fermentation mode of swine manure composting. It was found that the level of airborne bacteria in the swine manure composting facility was relatively high, compared with the concentration of airborne bacteria measured in other types of indoor spaces, such as subways [16,17], facilities for sensitive people (hospital, kindergarten, elderly welfare facilities, and postpartum care centers) [18], offices [19], and feed manufacturing plants [9]. However, it was similar to the concentration of airborne bacteria in the swine house [20], where organic substances that act as a source of airborne bacteria, such as swine manure, exist.

The concentrations of airborne bacteria emitted from swine manure composting plants before, during, and after turning the compost pile were not significantly different in this study. This finding was different from the results of a previous report [11], where a large amount of microorganisms distributed in the compost pile were generally discharged into the air through turning. It is presumed that the reason for this result was that the air sampling time after turning was too long, at 1 h, which did not reflect the effect of turning.

There is one study case that reported the particle size distribution characteristics of specific microorganisms in the air through a chamber test under experimental conditions [21], but a comparison with the results obtained from this field study would be irrational. Instead, the results of previous studies that reported the distribution characteristics of airborne bacteria by particle size and targeting indoor spaces other than swine manure composting plants are as follows [9,18,22]. Kim and Kim [18] and Kim et al. [22], who studied multi-use facilities, reported that the concentration ratio of airborne bacteria was the highest in stage 5 (1.1–2.1 μm) and the respiratory particle size ratio to the total concentration was 30–40%. Kim et al. [9], who studied the particle size distribution characteristics of airborne bacteria in the working environment of a feed manufacturing factory, reported that the highest ratio was in stage 5 (1.1–2.1 μm) and the lowest was in stage 3 (3.3–4.7 μm), and the respiratory concentration ratio to the total concentration was about 40%. When compared with this measurement result, the distribution characteristics by particle size were generally similar, but the respiratory concentration ratio was found to be relatively high.

As a result of statistical analysis, it was found that there were no internal environmental factors showing a significant correlation with the airborne bacteria generated in the swine manure composting plants. However, since researchers have different opinions on how temperature and relative humidity affect the generation of airborne bacteria [23–26] and this statistical analysis was performed based on the results of relatively few measured samples, the objective reliability was judged to be low. Therefore, in order to draw a clear scientific conclusion, it is considered that further studies should be made in the future. In general, particulate matters are known to move in the air by adsorbing gaseous substances or airborne microorganisms on their surfaces [27], so it can be assumed that particulate matter and airborne bacteria show a positive correlation. However, in this study, it was peculiar that particulate matter showed a positive correlation with gaseous odor ($p < 0.01$), but no significant positive correlation with airborne bacteria ($p > 0.05$).

As a result, the species profile of airborne bacteria in swine manure composting plants was generally similar, regardless of fermentation mode. This finding can be attributed to use of the same resource, namely swine manure, as composting material. *Micrococcus* spp. and *Staphylococcus* spp. are the predominant species of airborne bacteria found in other indoor facilities [18,22,28–30], which is generally identical to the airborne bacterial profile in the swine manure composting plants investigated in this study. A peculiar finding compared to previous reports is that *Enterobacteriaceae* spp., *Enterococcus* spp., and *Escherichia (E-coli)* as kinds of enteric bacteria constituted approximately 30% of all the airborne bacteria identified in the swine manure composting plants. This can also be explained by the fact that the composting material was manure excreted from the intestines of swine.

However, the qualitative methodologies for identifying airborne bacteria applied in this study were classical methods and only complementary to those provided by molecular techniques such as 16S rRNA sequencing. Thus, this study has a limitation in that the identification data obtained are uncertain and therefore the bacteria identified may be a health hazard.

5. Conclusions

The mean concentrations of airborne bacteria measured at swine manure composting plants were 7428 ($\pm$1024) CFU m^{-3} for the screw type, 3246 ($\pm$1407) CFU m^{-3} for the rotary type, and 5232 ($\pm$1217) CFU m^{-3} for the natural dry type. The swine manure composting plant of the screw type showed the highest concentration of airborne bacteria, followed by the natural dry type, and rotary type. The monthly level of airborne bacteria was highest in August and lowest in November, regardless of fermentation mode. The ratio of respirable size to total airborne bacteria was approximately 500%. There were no significant correlation relationships between airborne bacteria and environmental factors, such as temperature, relative humidity, and particulate matters in the swine manure composting plants. The predominant genera of airborne bacteria identified were *Micrococcus* spp., *Staphylococcus* spp., *Escherichia (E-coli)* spp., *Enterococcus* spp., *and Enterobacteriaceae* spp.

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