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Microbial community structure and distribution in the air of a powdered infant formula factory based on cultivation and high-throughput sequence methods

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ABSTRACT

The air in a powdered infant formula (PIF) factory is a potential transfer medium for microorganisms. In this study, air samples from 6 main processing areas, almost covering the whole PIF processing line and 1 outdoor location, were collected from a PIF manufacturing plant during the winter and summer periods. A cultivation-based and an Illumina (San Diego, CA) high-throughput 16S rRNA sequencing method was used to investigate the community structures and distributions of bacteria in the air. High microbial diversity (25 genera, 56 species), with a dominant community including *Staphylococcus*, *Bacillus*, *Acinetobacter*, and *Kocuria*, was found by the cultivation-based method. Moreover, 104 genera were obtained from all samples by high-throughput sequencing methods. *Lactococcus* (32.3%), *Bacillus* (29.6%), and *Staphylococcus* (14.0%) were the preponderant genera. The indices from high-throughput sequencing results indicated that the bacterial community of the air samples was highly diverse. Significant differences in the diversity and distribution at 6 sampling locations were revealed using the 2 methods. In particular, the packaging process contained the highest proportion (39.4%) of isolated strains. The highest diversity in bacterial community structure was found in the outdoor location. More bacterial isolates and higher community diversity were observed in the summer samples compared with the winter samples. In addition, some pathogens, such as *Acinetobacter baumannii*, *Bacillus cereus*, and *Staphylococcus cohnii*, were mainly found in the large bag filling process, can filling, and packaging process areas. The present study provides greater insight into the microbial community and identifies potential sources of air contamination in PIF production environments and can serve as a guide to reduce the risk of microbial contamination in the production of PIF.

Key words: airborne microorganism, bacterial diversity, powdered infant formula, milk, high-throughput sequencing

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INTRODUCTION

Powdered infant formula (**PIF**) is an effective breast milk substitute, providing all or most of the nutritional requirements for the normal growth and development of infants (Kent et al., 2015). However, PIF is not considered to be a sterile product, which has caused a potential safety risk to infants' health (Mullane et al., 2007, 2008). *Cronobacter* spp. (*Enterobacter sakazakii*), an important potential pathogen for neonates and infants, has been detected in PIF, dried baby foods, milk powder, and the production environment of PIF (Iversen and Forsythe, 2004; Sonbol et al., 2013; Fei et al., 2015). In a study conducted in Malaysia, it was reported that 13% of PIF, follow-up formulas, and infant foods samples (n = 90) had viable counts higher than 10^4 cfu/g. Aerobic plate counts of PIF samples ranged between 10^2 and 7.3×10^3 cfu/g. The most frequent *Enterobacteriaceae* isolated was *Enterobacter* spp., followed by *Citrobacter* spp. and *Klebsiella* spp. (Abdullah Sani et al., 2013). Various methods have been developed for the detection and identification of microbial hazards in infant foods, including real-time PCR (Fricker-Feer et al., 2011), probe-magnetic beads (Xu et al., 2014), and matrix-assisted laser desorption/ionization time-of-flight MS (Lu et al., 2014). Aflatoxin M_1, a carcinogenic mycotoxin, has also been found in infant food (Kabak, 2012). The presence and levels of aflatoxin M_1 contamination were investigated in 34 infant formula samples, with only 1 positive sample detected at a concentration of 0.0061 μg/kg (Er et al., 2014). Besides the risk of intrinsic microbial contaminants, poor hygienic practices and the use of inadequately sanitized utensils can cause the extrinsic contamination by pathogenic bacteria during PIF reconstitution (Araújo et al., 2015;

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Rachon et al., 2017). Therefore, reducing the microbial contamination of PIF is of considerable importance to neonatal health.

In the food manufacturing environment, air can be considered to be a transfer medium for the dissemination of bioaerosols, with airborne microorganisms including bacteria, fungi, viruses, pollen, and microscopic protozoa (Stetzenbach et al., 2004; Lee, 2011). Air contamination through poor environmental conditions can enable pathogens to be spread widely with air currents (Eduard et al., 2012). Consequently, airborne pathogens may be in contact with final products, equipment surfaces, and containers and result in PIF contamination during the production process (Brandl et al., 2014). Bacteria associated with PIF contamination, especially *Enterobacteriaceae*, have been isolated from the air of PIF processing environments (Angulo et al., 2008; Jacobs et al., 2011). Previous studies have proposed that the food-borne pathogenic bacteria suspended in the air could cause severe neonatal infections through contamination of the final products (Proudy et al., 2008; Yan et al., 2012). Jacobs et al. (2011) reported the high recovery of the neonatal pathogen *Cronobacter* from airborne dust collected by the air filters. The volume of dust was considerable and had been fed back into the production line. Thus, airborne bacteria are regarded as a potential contamination source in PIF processing and may result in product quality and safety issues.

Brandl et al. (2014) demonstrated the distribution of microorganisms in the air of a Swiss milk processing facility using the traditional cultivation-based method. Ninety-four morphologically different colony types were identified from the air of different sampling locations. These isolates included opportunistic pathogens, such as *Roseomonas mucosa*, *Bacillus cereus*, *Brevundimonas diminuta*, *Moraxella osloensis*, *Macrococcus caseolyticus*, *Staphylococcus cohnii*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. Most of the opportunistic pathogens were isolated from the processes of tin filling and bag filling. However, not all the opportunistic pathogens can be cultured. In previous studies, the majority of airborne microorganisms were unculturable, with only 0.01 to 10.0% of microorganisms able to be detected by cultivation-based methods (Horner-Devine et al., 2004; Riesenfeld et al., 2004). Consequently, the structure of microbial communities in the air cannot be completely determined using conventional cultivation methods. To overcome this shortcoming, high-throughput sequencing technology can be used to provide more detailed information concerning microbial community structures. At present, this technology has been used to analyze environmental biodiversity in many fields, such as wastewater treatment and soil (Roesch et al., 2007; Hu et al., 2012; Poulsen et al., 2013). Moreover, the microbial community composition of the air of a PIF factory is expected to be highly diverse. Therefore, determining the airborne microbial community structure within a PIF factory is warranted.

In this study, air samples were collected from 6 locations (5 indoor locations and 1 outdoor location) during winter and summer periods. These covered the entire PIF processing line. The microbial community structures of the air samples obtained from a typical PIF factory were investigated using both cultivation-based and Illumina (San Diego, CA) high-throughput sequencing methods. Our study aimed to provide a more comprehensive understanding of the microbial community structure in the air of a PIF factory, which is of relevance to microbial source tracking of air contamination during the production process.

MATERIALS AND METHODS

Sampling Locations

This study was conducted in a typical wet-processing PIF factory located in the northeast of China. A flowchart of the PIF process is shown in Figure 1. Six sampling locations (A–F) were selected in this study. Indoor sampling locations covered the whole processing line, including pretreatment (A), production (B), large bag filling (C), can filling (D), and packaging (E). An outdoor sampling location (F) was used as the reference.

Sample Collection

The air samples were collected at each sampling site during the same period between 0800 and 1700 h in 2 different seasons, winter (November to December 2014) and summer (June to July 2015). A JWL-SW1 microbe air sampler (Sennon, Beijing, China) with a flow rate of 200 L/min was used for collecting airborne bacteria. During each sampling, the sampler was mounted on a tripod at a height of about 1 m above the floor. To reduce environmental contamination, before sampling the impactor was disassembled and irradiated with UV radiation for 1 h. Between each sampling, the disc in contact with air and the surface of the sampler were wiped with 75% (vol/vol) ethanol solution for 3 min (Wang et al., 2010; Leung et al., 2014). The impactor was sealed when not in use.

Cultivable samples were collected on 90-mm trypticase soy agar (**TSA**) plates with 3 repetitions for each time point. Sampling time was 2.5 min, and impaction volume was 500 L. The samples were immediately incubated at 37°C for 3 to 7 d after collection (Zollinger et al., 2006). The amount of sampling was dependent

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