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Distribution and identification of culturable airborne microorganisms in a Swiss milk processing facility

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ABSTRACT

Airborne communities (mainly bacteria) were sampled and characterized (concentration levels and diversity) at 1 outdoor and 6 indoor sites within a Swiss dairy production facility. Air samples were collected on 2 sampling dates in different seasons, one in February and one in July 2012 using impaction bioaerosol samplers. After cultivation, isolates were identified by mass spectrometry (matrix-assisted laser desorption/ionization-time-of-flight) and molecular (sequencing of 16S rRNA and *rpoB* genes) methods. In general, total airborne particle loads and total bacterial counts were higher in winter than in summer, but remained constant within each indoor sampling site at both sampling times (February and July). Bacterial numbers were generally very low (<100 cfu/m^3 of air) during the different steps of milk powder production. Elevated bacterial concentrations (with mean values of 391 ± 142 and 179 ± 33 cfu/m^3 of air during winter and summer sampling, respectively; n = 15) occurred mainly in the "logistics area," where products in closed tins are packed in secondary packaging material and prepared for shipping. However, total bacterial counts at the outdoor site varied, with a 5- to 6-fold higher concentration observed in winter compared with summer. Twenty-five gram-positive and gram-negative genera were identified as part of the airborne microflora, with *Bacillus* and *Staphylococcus* being the most frequent genera identified. Overall, the culturable microflora community showed a composition typical and representative for the specific location. Bacterial counts were highly correlated with total airborne particles in the size range 1 to 5 μm, indicating that a simple surveillance system based upon counting of airborne particles could be implemented. The data generated in this study could be used to evaluate the effectiveness of the dairy plant's sanitation program and to identify potential sources of airborne contamination, resulting in increased food safety.
Key words: milk powder, airborne particles, bioaerosol, milk powder processing

INTRODUCTION

Exposure to microbially contaminated surfaces has been identified as the main source for food contamination (Otto et al., 2011). Recently, however, contamination of products by airborne microorganisms has been addressed (Shale and Lues, 2007). Improper sanitary environmental conditions in food processing plants can occur because of suspended biological particles in the air (Sutton, 2004). Therefore, in milk powder and powdered infant formula (**PIF**) processing facilities, the environmental air intake is strictly controlled (e.g., by the installation of air intake filters and the maintenance of overpressure), especially in high-risk areas. However, (re)-contamination of the products after the last heat treatment; for example, during filling and packaging, must be prevented.

Airborne contaminants of biological origin are microscopic, with diameters of 0.5 to 50 μm, and are known as bioaerosols, which may include bacteria, fungi, viruses, and pollen (Stetzenbach et al., 2004; Lee, 2011). Bioaerosols are easily translocated by winds and air currents from one ecosystem to another, making them an important vehicle for the spread of potentially pathogenic organisms (Wijnand et al., 2012). When associated with dust particles or condensation droplets, these organisms can be dispersed in a food processing unit. They may come in contact with food products, equipment, containers, and other food contact surfaces during handling. The food industry is required by the authorities (e.g., the US Food and Drug Administration) to take measures to reduce product contamination with airborne microorganisms (Heldman, 1974; Downes and Ito, 2001).

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Bioaerosols occur as droplets or solid particles and derive from a multitude of natural and artificial sources, such as surface waters, dry soils, agricultural activities, or food processing in the form of environmental dust during powder production (Zollinger et al., 2006; Mandal and Brandl, 2011). Air serves as the transport medium for the dispersal of bioaerosols. Transport and ultimate settling are affected by the physical properties of the aerosol particles (e.g., size, density, and shape) as well as environmental parameters (e.g., air currents, humidity, or temperature; Chao et al., 2002).

It is impossible to keep airborne bacteria, yeasts, and molds at a zero level in food processing. In dairy production facilities, general handling of ingredients, spray drying, and milling operations can create bioaerosols (Mullane et al., 2008; Dungan et al., 2010; Dungan, 2012). Wet and dry cleaning operations often result in the formation of bioaerosols in the form of water droplets or dry dust originating from sweeping or directly from the exhaust of vacuum cleaners (Abt et al., 2000). However, standard practice in milk processing and PIF production include the use of high-efficiency particulate air (HEPA) filters for exhaust air of all vacuum cleaners to maintain a high hygienic level (Kandhai et al., 2004; Mullane et al., 2008; Iversen et al., 2009; Jacobs et al., 2011). Moreover, proper selection and maintenance of air filters is crucial to control the input of airborne contaminants into processing steps and ultimately to exclude health hazards from the final products (Liu et al., 2009).

In the present study, we performed a detailed study on airborne particle concentration measurements combined with enumeration and characterization of total culturable airborne bacteria and yeast and mold contaminants within a Swiss dairy processing facility. Samples were collected at 7 defined locations on 2 sampling dates in different seasons. The study was intended as a baseline study to establish the status quo and set the background for future work; for example, investigating the influence of environmental parameters such as temperature, humidity, or time of the year. Results presented here provide basic data on concentration levels and size distribution of airborne particles as well as on the variability of airborne organisms in various environments throughout the milk processing line.

MATERIALS AND METHODS

Milk Processing Facility and Selection of Sampling Sites

The study was conducted at the milk powder processing unit of a dairy plant located in Switzerland. Figure 1 represents a flowchart of the processing scheme for milk powder and PIF production. Sampling sites selected for the study were designated P1 to P7 (Table 1); P1 to P6 were indoor sites and P7 represented an outdoor sampling site. Air samples were collected on 2 sampling dates, one in February and one in July 2012.

Air samples were collected from P1 to P7 within the milk processing line representing different hygienic zones (Figure 1). The dairy processing unit was divided into highly hygienic areas (white zone), hygienic areas (blue zone), and logistics areas (red zone; lower hygienic standard). Highly hygienic areas included sites P1, P2, and P3 (production zones where contamination must be avoided), and hygienic areas included sites P5 and P6. Site P4 was located in the red zone in the logistics area. One outdoor location (P7) was chosen as reference. Fifteen 100-L air samples were taken simultaneously at each sampling site for the determination of culturable bacteria.

Particle Counting

Three handheld laser particle counters (MetOne model 227B, Skan AG, Allschwil, Switzerland) were used to determine particle numbers (Zollinger et al., 2006). In total, particles of 4 size classes (0.3–0.5 μm, >0.5–1 μm, >1–5 μm, and >5 μm) were measured. To assess experimental standard errors, particles ranging from 0.3 to 0.5 μm were counted in triplicate. Generally, variations between the 3 instruments were <4% (Zollinger et al. 2006).One of the particle counters was equipped a sensor to monitor temperature and relative humidity. Depending on the sampling location (Table 1), between 9 and 23 air samples (1 L each) were taken at regular intervals of 1.5 min during the whole monitoring period. Two different particle size classes were simultaneously recorded with each instrument. Readings were stored in the internal memory of the instrument and subsequently analyzed using the software Particle Vision PortAll 1.2 (Zollinger et al., 2006). Linear regression was performed with SigmaPlot (version 8; Systat Software, San Jose, CA).

Impaction Air Sampling and Cultivation

Three MAS-100 Eco impaction samplers (MBV, Stäfa, Switzerland) were used to collect bioaerosols onto solid agar surfaces (Zollinger et al., 2006). Generally, 100-L samples were collected in time intervals of approximately 3 min. At each location (see Table 1), fifteen 90-mm Petri dishes containing standard growth medium were used with the impaction sampler. Plate count agar (PCA) containing (in g/L) casein peptone (5.0), yeast extract (2.5), glucose (1.0), and agar (9.0) was used to determine the total number of cultur-