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# Spore populations among bulk tank raw milk and dairy powders are significantly different

**Rachel A. Miller, David J. Kent, Matthew J. Watterson, Kathryn J. Boor, Nicole H. Martin, and Martin Wiedmann[1]**
Milk Quality Improvement Program, Department of Food Science, Cornell University, Ithaca, NY 14853

## ABSTRACT

To accommodate stringent spore limits mandated for the export of dairy powders, a more thorough understanding of the spore species present will be necessary to develop prospective strategies to identify and reduce sources (i.e., raw materials or in-plant) of contamination. We characterized 1,523 spore isolates obtained from bulk tank raw milk (n = 33 farms) and samples collected from 4 different dairy powder-processing plants producing acid whey, nonfat dry milk, sweet whey, or whey protein concentrate 80. The spores isolated comprised 12 genera, at least 44 species, and 216 *rpoB* allelic types. *Bacillus* and *Geobacillus* represented the most commonly isolated spore genera (approximately 68.9 and 12.1%, respectively, of all spore isolates). Whereas *Bacillus licheniformis* was isolated from samples collected from all plants and farms, *Geobacillus* spp. were isolated from samples from 3 out of 4 plants and just 1 out of 33 farms. We found significant differences between the spore population isolated from bulk tank raw milk and those isolated from dairy powder plant samples, except samples from the plant producing acid whey. A comparison of spore species isolated from raw materials and finished powders showed that although certain species, such as *B. licheniformis*, were found in both raw and finished product samples, other species, such as *Geobacillus* spp. and *Anoxybacillus* spp., were more frequently isolated from finished powders. Importantly, we found that 8 out of 12 genera were isolated from at least 2 different spore count methods, suggesting that some spore count methods may provide redundant information if used in parallel. Together, our results suggest that (1) *Bacillus* and *Geobacillus* are the predominant spore contaminants in a variety of dairy powders, implying that future research efforts targeted at elucidating approaches to reduce levels of spores in dairy powders should focus on controlling levels of spore isolates from these genera; and (2) the spore populations isolated from bulk tank raw milk and some dairy powder products are significantly different, suggesting that targeting in-plant sources of contamination may be important for achieving low spore counts in the finished product. These data provide important insight regarding the diversity of spore populations isolated from dairy powders and bulk tank raw milk, and demonstrate that several spore genera are detected by multiple spore count methods.

**Key words:** spore populations, dairy powders, bulk tank raw milk

## INTRODUCTION

Bacterial endospores (referred to here as spores) represent important quality and safety indicators for dairy powders (Burgess et al., 2010; Watterson et al., 2014; Hwang and Park, 2015). Their resistance to several environmental stresses, including high temperature, low pH, and low water activity, makes spores well suited to survive the various environments encountered during dairy powder production (Setlow, 2006; Burgess et al., 2010; Witthuhn et al., 2011).

Previous accounts of aerobic spore populations in dairy powders were restricted to nonfat dry milk (NDM) and whole milk powder (**WMP**). For these dairy powders, *Anoxybacillus flavithermus*, *Geobacillus stearothermophilus*, and *Bacillus licheniformis* represent the most frequently isolated organisms (Scott et al., 2007; Burgess et al., 2010; Yuan et al., 2012). However, *A. flavithermus* and *G. stearothermophilus* are rarely isolated from bulk tank (**BT**) raw milk, where the predominant spore species include *B. licheniformis*, *Bacillus pumilus*, and *Bacillus subtilis* (Coorevits et al., 2008; Burgess et al., 2010; Yuan et al., 2012). The disparity in spore populations isolated from dairy powders and BT raw milk is often attributed to in-plant sources of contamination. Scott et al. (2007) isolated *A. flavithermus* and *Geobacillus* spp. from multiple sampling points in a WMP processing plant. Those authors noted that *A. flavithermus* was the predominant organism in preheating processing steps, but both *Geobacillus* spp. and *A. flavithermus* were isolated at subsequent processing

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[1]Corresponding author: mw16@cornell.edu

steps (Scott et al., 2007). This demonstrates the dynamic nature of spore populations in dairy powders and highlights the need for an improved understanding of the spore species, which are associated with raw material and in-plant contamination.

Currently, no public reports exist of spore populations in dairy powders, such as whey protein concentrate 80 (**WPC-80**), sweet whey (**SW**), and acid whey (**AW**); this would be of significant economic value to dairy powder exporters due to customer requirements for powders to meet strict spore count limits. Therefore, it is important to identify the spores present in these dairy powders so that preventative approaches to limiting spore contamination in these products can be developed.

Previous studies have identified contamination originating from biofilms formed by spore-forming bacteria as an important source of bacterial spores in dairy powders (Parkar et al., 2001; Scott et al., 2007; Burgess et al., 2010). In favorable environments, spores are capable of germinating into bacterial cells, which are then able to adhere to processing equipment and, subsequently, establish biofilms. Additionally, the heat tolerance of several spore species suggests that they are capable of surviving in the product throughout processing (te Giffel et al., 2002; Scheldeman et al., 2005; Burgess et al., 2010). For these reasons, mandating low spore levels in raw materials has been identified as an important approach to reducing spore loads in the finished product (Watterson et al., 2014). However, few studies have examined differences in spore populations isolated from raw materials and finished products (Scott et al., 2007; Watterson et al., 2014). An improved understanding of the processing steps at which spores contaminate dairy powders would provide relevant information that would be useful for developing enhanced control strategies.

Spore levels may be quantified using a variety of spore count methods (**SCM**), each requiring different temperature and time combinations [referred to as spore pasteurization (**SP**)] to eliminate vegetative bacterial cells, as well as different incubation temperatures and plating media to enumerate the resulting spore-forming bacteria (Ronimus et al., 2003; Bienvenue, 2014; Watterson et al., 2014). For example, the standard spore pasteurization (**SSP**) method requires a heat treatment of 80°C for 12 min, whereas the highly heat resistant (**HHR**) SCM uses a heat treatment of 100°C for 30 min (Frank and Yousef, 2004; Watterson et al., 2014). Currently, it is unknown how these different SCM may influence the resulting spore count of the product tested or the composition of the spore populations recovered. Work by Watterson et al. (2014) suggests differences exist in the resulting spore counts obtained by HHR and SSP methods, but it is unknown how these methods affect the resulting spore species that are isolated. Because dairy powder customers may require that multiple SCM be performed to assess spore levels in a dairy powder, it is important to determine the spore species that may be detected or excluded by different SCM.

Therefore, the goals of this study were to (1) characterize the spore populations of a variety of dairy powder (AW, NDM, SW, and WPC-80) and BT raw milk sources; (2) determine differences in spore populations isolated from raw materials and finished products; and (3) determine which spore genera are typically detected by different SCM.

## MATERIALS AND METHODS

### *Description of Spore Isolates Used in This Study*

A total of 1,949 bacterial isolates, collected from BT raw milk and dairy powder samples that had been SP, were characterized in this study. Isolates were obtained from 2 previous studies examining spore counts in (1) BT raw milk from 33 different farms throughout New York State sampled every other month for 1 yr (i.e., 6 BT raw milk samples per farm, total of 198 samples; Miller et al., 2015); and (2) 4 different dairy powder processing plants sampled every other month for 1 yr (i.e., 6 sampling dates per plant, total of 147 samples from plants processing AW, NDM, SW, or WPC-80; Watterson et al., 2014). Samples collected in the 4 plants included (1) raw materials (e.g., raw milk, whey powders, liquid whey), (2) product collected at intermediate processing stages (work in process; **WIP**), and (3) finished product (see Table 1; Watterson et al., 2014). All samples had been SP by heating at 80°C for 12 min for SSP, or 100°C for 30 min for HHR SP (only performed for dairy powder plant samples), followed by incubation at 6°C for 10 d [psychrotolerant spore count (**PSC**)] to enumerate psychrotolerant spores, and at 32° and 55°C for 48 h to enumerate mesophilic [mesophilic spore count (**MSC**)] and thermophilic [thermophilic spore count (**TSC**)] spores, respectively (Frank and Yousef, 2004). An additional enrichment step, in which SP samples were incubated at either 6°C for 10 d, or at 32 or 55°C for 48 h, followed by plating on brain-heart infusion (**BHI**) agar and subsequent incubation at 6°C for 10 d, or at 32 or 55°C for 48 h, was done to detect spores present at levels below detection (present at levels <10 cfu/mL). Morphologically distinct colonies obtained from PSC, MSC, and TSC analyses had been substreaked onto BHI plates, followed by incubation at 6, 32, or 55°C, depending on the original temperature of isolation. Isolated colonies from substreaked plates had been propagated in BHI broth (Difco; Franklin