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Evaluation of dairy powder products implicates thermophilic sporeformers as the primary organisms of interest

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ABSTRACT

Dairy powder products (e.g., sweet whey, nonfat dry milk, acid whey, and whey protein concentrate-80) are of economic interest to the dairy industry. According to the US Dairy Export Council, customers have set strict tolerances (<500 to <1,000/g) for thermophilic and mesophilic spores in dairy powders; therefore, understanding proliferation and survival of sporeforming organisms within dairy powder processing plants is necessary to control and reduce sporeformer counts. Raw, work-in-process, and finished product samples were collected from 4 dairy powder processing facilities in the northeastern United States over a 1-yr period. Two separate spore treatments: (1) 80°C for 12 min (to detect sporeformers) and (2) 100°C for 30 min (to detect highly heat resistant sporeformers) were applied to samples before microbiological analyses. Raw material, work-in-process, and finished product samples were analyzed for thermophilic, mesophilic, and psychrotolerant sporeformers, with 77.5, 71.0, and 4.6% of samples being positive for those organisms, respectively. Work-in-process and finished product samples were also analyzed for highly heat resistant thermophilic and mesophilic sporeformers, with 63.7 and 42.6% of samples being positive, respectively. Sporeformer prevalence and counts varied considerably by product and plant; sweet whey and nonfat dry milk showed a higher prevalence of thermophilic and mesophilic sporeformers compared with acid whey and whey protein concentrate-80. Unlike previous reports, we found limited evidence for increased spore counts toward the end of processing runs. Our data provide important insight into spore contamination patterns associated with production of different types of dairy powders and support that thermophilic sporeformers are the primary organism of concern in dairy powders.

Key words: thermophilic, mesophilic, sporeformer, milk powder

INTRODUCTION

Sporeforming organisms have been detected throughout the dairy processing continuum (Crielly et al., 1994; Postollec et al., 2012), including dairy farm environments, storage and transportation tanks, and dairy processing plants. On the dairy farm, sporeforming organisms have been isolated from soil and teats (Christiansson et al., 1999); pasture (Slaghuis et al., 1997); bedding, silage, and feed (Crielly et al., 1994; te Giffel et al., 2002; Magnusson et al., 2007); fecal material (Labots et al., 1965; Huck et al., 2008); and raw milk (Boor et al., 1998; Huck et al., 2007; Martin et al., 2011). Sporeforming organisms have been detected in milk samples collected during intermediate storage and transportation, including in bulk tank raw milk (Griffiths and Phillips, 1990; Crielly et al., 1994) and in raw milk in transport tankers (Huck et al., 2007). Sporeformers have also been isolated from the environment of dairy product processing plants (Ralyea et al., 1998; Fromm and Boor, 2004; Huck et al., 2007) and have been detected in ready-to-eat pasteurized dairy products (e.g., fluid milk; Fromm and Boor, 2004; Huck et al., 2007); in Gouda and semi-hard cheeses (Klijn et al., 1995); and in dairy powder products (Murphy et al., 1999; Ronimus et al., 2003; Scott et al., 2007; Burgess et al., 2009, 2010).

Aerobic sporeformers of particular concern in dairy products include the psychrotolerant *Paenibacillus* spp. (Fromm and Boor, 2004; Huck et al., 2007; Ranieri et al., 2009; Ivy, et al., 2012), mesophilic *Bacillus* spp.; for example, *Bacillus licheniformis*, *Bacillus subtilis*, and *Bacillus pumilus* (Crielly et al., 1994; Fromm and Boor, 2004), and thermophilic *Anoxybacillus flavithermus* and *Geobacillus* (Burgess et al., 2009). The psychrotolerant sporeformers, such as *Paenibacillus* spp., are known to reduce shelf-life and cause spoilage of fluid milk products (Fromm and Boor, 2004; Durak et al., 2006; Huck et al., 2007; Ranieri et al., 2009). Mesophiles can cause shelf-life or keeping quality problems in shelf-stable milk products (Ridgway, 1954; Ridgway, 1955; Franklin et al., 1956). Thermophiles, such as *A. flavithermus* and *Geobacillus* spp. are known to cause quality and shelf-life concerns in products manufactured using dairy powders as ingredients (Muir et al., 1986; Flint et al.,

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1997). Other members of the *Bacillus* genus have also been known to cause quality defects in UHT milk and retorted products (Klijn et al., 1997; Scheldeman et al., 2006). *Clostridium tyrobutyricum*, a sporeformer and obligate anaerobe, is known to cause late-blowing defects in Gouda and other cheeses during aging (Klijn et al., 1995; Quiberoni et al., 2008). Quality defects that arise during the shelf life of dairy products result in food waste and create economic losses for dairy product manufacturers.

Sporeforming bacteria can survive the conditions found in food processing facilities because of their innate ability to resist adverse conditions by entering a resilient, dormant state through the formation of endospores. Spores ensure survival of the organism, as they have the ability to withstand arduous environmental conditions, including reduced nutrient availability, pH extremes, adverse temperatures, and reduced moisture conditions (De Vos et al., 2009). Within food production environments, spores survive the high temperatures used to cook food products or to pasteurize milk (Collins, 1981), and they resist the chemicals used to clean and sanitize equipment, surfaces, and utensils (Russell, 1990; Bloomfield and Arthur, 1994). When the environment returns to favorable conditions, the endospores activate, germinate, and return to the vegetative cell state through outgrowth, which is then followed by reproduction (De Vos et al., 2009).

As an additional survival mechanism, many bacteria—including sporeformers—can form biofilms, which further enhances their ability to persist under adverse environmental conditions (Zottola and Sasahara, 1994; Bower et al., 1996). Biofilm formation can be particularly problematic in food processing (Zottola and Sasahara, 1994; Kumar and Anand, 1998) and dairy processing environments (Flint et al., 1997; Faille et al., 2001; Burgess et al., 2009), as biofilms provide protection to vegetative cells and their spores from the biocidal effects of cleaning and sanitizing agents (Hood and Zottola, 1995; Kumar and Anand, 1998).

Vegetative cells and spores of sporeforming organisms (e.g., *Geobacillus*, *Anoxybacillus*, *B. licheniformis*, *Bacillus coagulans*, and *B. pumilis*) are of particular concern to the dairy industry because *Bacillus* spp. have been shown to adhere strongly to stainless steel (Flint et al., 1997; Parkar et al., 2001; Palmer et al., 2010), which is commonly found in dairy processing facilities, and to stainless steel with milk foulant (Flint et al., 2001a). Strong adhesion of the bacterial cells and spores to stainless steel surfaces further enhances biofilm development (Zottola and Sasahara, 1994; Kumar and Anand, 1998). Spores and vegetative cells that have become attached in biofilms may eventually detach and contaminate equipment downstream and the finished product (Hood and Zottola, 1995; Bower et al., 1996; Flint et al., 1997; Burgess et al., 2009), which can lead to quality defects and reduced shelf life of manufactured dairy products (Wong et al., 1988; Marchand et al., 2012).

During a previous study, Murphy et al. (1999) isolated *Bacillus stearothermophilus* (now *Geobacillus stearothermophilus*) and *B. licheniformis* from the tubular preheater(s) and the evaporator in a milk powder processing facility. More recently, Scott et al. (2007) isolated *A. flavithermus* and *Geobacillus* spp. from the preheater (plate heat exchanger) and evaporator in a milk powder processing plant. It is widely held within the dairy processing industry that sporeformer counts in dairy product powders increase as the production run increases, whereby longer runs produce higher counts. Murphy et al. (1999) reported increased growth in the evaporators within 4 h and significant contamination of the evaporator system after 8 h. Scott et al. (2007) found that sporeformer counts increased at both the preheater and evaporator steps between hour 9 and 18 of a whole milk powder processing run. These increases have been attributed to the contamination of the product from biofilm build-up and the sloughing off of foulant.

Recently, customers have set strict specifications for mesophilic and thermophilic sporeformers. For example, sporeformer specifications from international customers for dairy powders have been reported as follows: aerobic mesophilic and thermophilic spore counts <500 to <1,000 cfu/g for skim milk powder (**SMP**), NDM and whole milk powder (**WMP**) destined for infant formula; and <500 to <2,000 cfu/g for aerobic thermophilic spores in SMP and WMP destined for recombined or UHT products (Bienvenue, 2013). These stringent sporeformer specifications are very difficult to achieve and they present an important challenge to the dairy industry worldwide.

Reduction of sporeformer counts in finished dairy powder products to meet strict customer specifications requires a systematic approach to understanding the sources and niches that contribute to contamination of milk products with sporeforming organisms, from the farm, through distribution, and during processing. To this end, we surveyed 4 US dairy powder products over a 12-mo period to determine the prevalence and in-plant contamination patterns for psychrotolerant, mesophilic, and thermophilic sporeformers.

MATERIALS AND METHODS

Dairy Powder Processing Plants

Four dairy powder product processing facilities (plants A, B, C, and D; Table 1), located in the northeastern