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

United States, voluntarily participated in the yearlong study. Each of the 4 plants manufactures one of the following dairy powder products: (1) sweet whey powder (**SWP**; plant A); (2) NDM (plant B); (3) acid whey (**AW**; plant C); and (4) whey protein concentrate-80 (**WPC-80**; plant D). The NDM plant used previously untreated raw milk as the sole raw ingredient; the 3 whey powder plants utilized whey by-products from cheese manufacturing operations as raw ingredient(s). Within the 4 processing facilities, production runs varied between 10 and 12 h for the shortest runs and 18 to 24 h for the longest.

Sampling Scheme and Collection

During the 12-mo sampling period, raw ingredients (**R**), work-in-process (**WIP**), and finished product (**FP**) samples were aseptically collected once every 2 mo from multiple sample points within each plant. Depending on processing parameters, or individual plant design and set-up, samples were collected at 4, 6, 7, or 9 sampling points along the production line (Table 1; Figure 1). Sample collection occurred at 3 time points: beginning (**B**; collected within 2 h of the processing run start-up), middle (**M**; collected within 2 h of the

Table 1. Summary of plant characteristics and sample points for 4 dairy powder processing facilities in the northeast United States

Plant	Product	No. of sample points (raw) ¹
A	Sweet whey	9 (4)
B	Nonfat dry milk	6 (1)
C	Acid whey	4 (1)
D	Whey protein concentrate-80	7 (1)

¹Number in parentheses indicates the number of different raw materials collected. Raw materials collected in plant A represented whey (liquid), milk/whey permeate (liquid), whey protein concentrate-80 (powder), and lactose (powder).

projected midpoint of the run), and end (**E**; collected within the last 2 h of production).

Raw ingredients collected at each time point (**B**, **M**, and **E**) included (1) raw milk; (2) whey by-products from cheese making (e.g., Cheddar, ricotta, mozzarella, and cottage cheese); (3) lactose powder; (4) WPC-80 powder; and (5) lecithin (liquid). Raw fluid samples were aseptically collected in 500-mL sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) from storage tanks or silos (Figure 1). Dry, raw ingredient samples were collected in 100-g portions and deposited into sterile Whirl-Pak (Nasco) bags, using sterile scoops, from aseptically opened bulk ingredient bags (only from

Figure 1. Consolidated flow diagram representing basic processing steps for all 4 processing plants; shaded boxes represent process steps that are not used in every plant process. Actual sample points, which varied between 4 and 9 sample points per plant, are represented by symbols: □ = sweet whey; ▲ = nonfat dry milk; ○ = acid whey; and ◆ = whey protein concentrate-80 (WPC-80). Sample points where more than one sample may have been collected at a given sampling time are marked with an asterisk (only applicable to select processes); for example, in some plants multiple raw ingredients (e.g., WPC-80, lactose, and lecithin) or more than one raw ingredient tank may have been sampled. RO = reverse osmosis.

those plants that used dry raw ingredients within the process).

The WIP samples (500 mL of fluid or 100 g of powder) were aseptically collected in sterile Whirl-Pak (Nasco) bags from intermediate locations along the production line at each time point (B, M, and E). The WIP sample points included (1) balance tank(s), (2) intermediate storage tank(s), (3) post-UF or reverse osmosis concentration, (4) post-evaporator, (5) condensed product silos, and (6) processing steps after the spray drier (e.g., at the sifter, slide, or belt) (Figure 1). The WIP samples were collected from intermediate holding tanks using washed and sanitized petcocks or valves, or sanitized dipping devices when necessary, depending on sample point location and plant configuration. When samples were collected using a petcock or valve, the device was chemically sanitized (e.g., with iodine or calcium hypochlorite), and the petcock or valve was flushed for 10 to 15 s to expel one-half to one gallon of fluid product, in accordance with *Standard Methods for the Examination of Dairy Products*, 17th ed. (SMEDP; Graham, 2004). Dry WIP samples were collected aseptically from conveyor belts using sterile scoops or from falling powders (e.g., at the slide after the spray-drier, or from the sifter; Figure 1), using suspended, sterile Whirl-Pak (Nasco) bags.

Finished product samples were aseptically collected using sterile scoops from FP bags at the bagger that were not yet sealed or aseptically opened FP bags. All samples were held at or below 6°C awaiting transport to the Milk Quality Improvement Program (MQIP) laboratory (Cornell University, Ithaca, NY). Samples were packed on ice in insulated coolers and shipped by overnight delivery or picked up at the processing plant by MQIP laboratory personnel within 24 h of sampling. Temperature controls were included in each cooler and were evaluated immediately upon sample arrival at the laboratory; samples with temperatures >6°C were rejected and replacement samples were collected later.

Spore Count Treatments, Plating and Enrichments

A spore pasteurization (SP) treatment (80°C for 12 min) was performed on all samples in accordance with SMEDP (Frank and Yousef, 2004) for enumeration of aerobic bacterial spores. Dry WIP and FP samples were rehydrated in phosphate buffer solution with magnesium chloride (Weber Scientific, Hamilton, NJ) at a ratio of 1:10 (wt/wt). Fluid product and rehydrated powder samples (100 mL) were placed in sterile, glass Pyrex (Corning Inc., Corning, NY) bottles, heated to 80°C for 12 min to inactivate all vegetative cells, and rapidly cooled to <6°C in an ice bath. All samples were spiral plated (Autoplate 4000, Spiral Biotech, Norwood,

MA), in duplicate, on brain heart infusion (BHI) agar for each of the following tests: (1) psychrotolerant spore count (PSC; incubated at 6°C for 10 d); (2) mesophilic spore count (MSC; incubated at 32°C for 48 h); and (3) thermophilic spore count (TSC; incubated at 55°C for 48 h). Additional dilution of certain samples was necessary after heat treatment and before spiral plating. Colonies were enumerated immediately following each incubation period.

Additionally, immediately following the spore treatment and subsequent cooling, 30-mL aliquots of each spore treatment were placed in 3 separate 60-mL vials to allow for enrichment that enabled detection of low levels of psychrotolerant, mesophilic, and thermophilic sporeformers. Enrichment vials were incubated under the following conditions: (1) PSC, 6°C for 10 d; (2) MSC, 32°C for 48 h; and (3) TSC, 55°C for 48 h. For those samples where the final count on direct plating was below the detection limit (i.e., no colonies present on BHI; Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7363>), a 10-μL aliquot of the enriched sample was streaked onto BHI and incubated as follows: (1) PSC enrichment, 6°C for 10 d; (2) MSC enrichment, 32°C for 48 h; and (3) TSC-enrichment, 55°C for 48 h (MSC and TSC enrichment plates were checked at 24 h for colony formation). After the incubation period, plates streaked from the enrichments were examined for presence or absence of visible colonies.

Evaluation of WIP and FP samples for highly heat resistant (HHR) spores was performed by heating both fluid and rehydrated powder (1:10 wt/wt in phosphate buffer) samples to 100°C for 30 min in a boiling water bath, and then rapidly cooling to <6°C before spiral plating, in duplicate, on BHI. In the same manner as for the SP samples, HHR-treated samples were tested for (1) MSC and (2) TSC, using the same incubation parameters as detailed above. Additional dilution of certain samples was necessary following heat treatment before spiral plating. After incubation, visible colonies were enumerated immediately, as described above. As detailed above for the SP treatment, 30-mL aliquots of HHR-treated samples were incubated at MSC and TSC parameters in individual 60-mL sterile vials to allow for the enrichment of low levels of HHR mesophilic and thermophilic sporeformers. A 10-μL aliquot of the enriched sample was streaked onto BHI if the direct-plated test (HHR MSC or HHR TSC) was below the detection limit (i.e., no colonies present on direct plating; Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7363>). Plates streaked from the enrichments after HHR treatment were incubated with the corresponding MSC and TSC incubation parameters detailed above and evaluated for presence of visible colonies.

Isolate Preservation

For each sample that displayed growth, on direct plating or after plating of enrichments, bacterial isolates were collected from BHI plates representing each test type (PSC, MSC, TSC, HHR MSC, and HHR TSC). Colonies representing each visually distinct morphology were selected and pure cultures were streaked for isolation. Single colonies were picked from the plates and streaked onto BHI agar. After 18 to 24 h of incubation (at 32°C for PSC, MSC, and HHR MSC, or 55°C for TSC and HHR TSC), single colonies were selected from the pure culture and inoculated into BHI broth. The samples in broth were incubated at the temperatures described above for 12 to 18 h. Aliquots were preserved in 15% glycerol and stored in duplicate at −80°C. Further information on isolates collected in this study can be found at www.foodmicrobetracker.net.

Statistical Analysis

Statistical analyses were performed in JMP (version 10.0, SAS Institute Inc., Cary, NC). Microbiological data were log-transformed before calculating means; both linear and logistic regressions were performed.

RESULTS AND DISCUSSION

Spore Populations in Dairy Powders

Overall, 4.6, 71.0, and 77.5% of samples were positive for psychrotolerant, mesophilic, or thermophilic spores, either by direct plating or enrichment. Utilizing the methods described, positive on direct plating indicates >10 cfu/mL or 100 cfu/g, and positive for enriched samples indicates >0.03 cfu/mL or >0.03 cfu/g, unless additional dilution of samples before plating was required (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7363>). Testing for PSC in samples from all 4 plants resulted in 1.7% of samples being positive after direct plating and an additional 2.9% being positive after enrichment (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7363>). The vast majority of samples (95.4%) thus showed no evidence of psychrotolerant sporeformers, even after enrichment (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7363>). This result is not surprising because the high temperatures of powder processing, typically in the 40 to 80°C range, should select for organisms that prefer to grow at higher temperatures. These results also imply that the likelihood of quality defects resulting from psychrotolerant sporeformers in finished powder products is relatively low. It should be noted, however, that despite the low incidence of psychrotoler-

ant sporeformers found in dairy powders in this study, previous work has demonstrated that extremely low levels of psychrotolerant sporeforming bacteria present in refrigerated fluid milk can result in quality defects and reduced shelf life (Ranieri et al., 2012), issues that remain a concern for fluid milk processors.

In contrast to the low incidence of psychrotolerant sporeformers, 37.8% of SP-treated samples were positive after direct plating for mesophilic sporeformers; an additional 33.2% of these samples were positive after enrichment (Table 2). Based on previous studies of dairy powders (Burgess et al., 2010; Yuan et al., 2012), the majority of these mesophilic organisms likely represent *B. licheniformis*, *B. subtilis*, and *B. pumilus* spores.

Finally, 59.4% of SP-treated samples were positive for thermophilic sporeformers at levels high enough to be detected on direct plating; an additional 18.1% of SP-treated samples were positive for thermophilic sporeformers after enrichment (Table 2). Based on previous studies, thermophilic sporeformers isolated from dairy powders typically include *A. flavithermus* and *Geobacillus* spp. (Scott et al., 2007); these organisms grow optimally at temperatures of 40 to 65°C (Flint et al., 2001b; Parkar et al., 2003; Scott et al., 2007). Murphy et al. (1999), Scott et al. (2007), and Seale et al. (2008, 2012) have previously isolated these organisms from preheaters (tubular and plate) and evaporator(s) in dairy processing powder environments. Combined with the high prevalence of thermophilic sporeformers we found here (77.5%), these data indicate that thermophilic sporeformers may be the primary organisms of concern for powder processors that try to meet stringent customer requirements for sporeformers counts.

Incidence of HHR Spores in Dairy Powders

After HHR treatment (100°C for 30 min), only 7.3% of all WIP and FP samples tested were positive by direct plating for HHR mesophilic sporeformers; an additional 35.3% of samples were positive after enrichment (Table 3). In contrast, after SP treatment (80°C for 12 min), 41.7 and 35.7% of SP-treated WIP and FP samples were positive for mesophilic sporeformers on direct plating, respectively; an additional 33.6 and 42.9% of WIP and FP samples were positive after enrichment (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7363>). The much lower frequency of samples positive for mesophilic sporeformers after HHR spore treatment, compared with SP treatment, was not unexpected given the severity of the HHR spore treatment, which is designed to select primarily for obligate thermophiles (Burgess et al., 2010).

After HHR, 32.8% of samples tested positive for thermophilic sporeformers by direct plating and 30.9% were

Table 2. Summary of thermophilic and mesophilic spore counts in dairy powders from 4 dairies in the northeast United States sampled 6 times over a 12-mo period

Plant (product)	Status ¹	Time point ² (no. of samples)	Thermophilic spore count (TSC)			Mesophilic spore count (MSC)		
			Samples positive (%)		Samples negative (%)	Samples positive (%)		Samples negative (%)
			After direct plating (mean log cfu/mL or /g)	After enrichment ³		After direct plating ⁴ (mean log cfu/mL or /g)	After enrichment	
A (sweet whey)	R	B (18)	78 (2.7)	6	16	44 (1.6)	17	39
	R	M (12)	83 (2.9)	17	0	17 (1.0)	42	41
	R	E (11)	91 (2.6)	9	0	9 (1.0)	55	36
	WIP	B (24)	100 (3.3)	0	0	33 (1.9)	33	34
	WIP	M (24)	100 (3.3)	0	0	21 (1.4)	50	29
	WIP	E (23)	100 (3.4)	0	0	39 (2.1)	52	9
	F	B (6)	100 (4.2)	0	0	17 (2.0)	67	16
	F	M (6)	100 (4.2)	0	0	17 (2.0)	83	0
	F	E (6)	100 (4.3)	0	0	33 (2.0)	67	0
Total/mean ⁵		130	94.6 (3.3)	3.1	2.3	28.5 (1.8)	45.4	26.1
B (NFDM)	R	B (6)	83 (1.6)	17	0	80 (1.6)	20	0
	R	M (6)	67 (1.3)	20	13	100 (1.6)	0	0
	R	E (6)	67 (1.5)	20	13	50 (1.4)	50	0
	WIP	B (24)	79 (2.1)	21	0	83 (1.9)	17	0
	WIP	M (24)	92 (2.2)	8	0	88 (1.9)	12	0
	WIP	E (22)	100 (3.2)	0	0	86 (2.0)	14	0
	F	B (6)	100 (2.6)	0	0	100 (2.1)	0	0
	F	M (6)	83 (2.3)	17	0	83 (2.2)	17	0
	F	E (6)	100 (3.3)	0	0	100 (2.3)	0	0
Total/mean		106	87.7 (2.4)	10.4	1.9	85.7 (1.9)	14.3	0.0
C (acid whey)	R	B (6)	17 (1.0)	0	83	NA ⁶	0	100
	R	M (6)	NA	0	100	17 (1.3)	17	66
	R	E (6)	NA	0	100	NA	0	100
	WIP	B (12)	8 (1.0)	8	84	25 (1.5)	17	58
	WIP	M (12)	33 (1.6)	8	59	42 (1.2)	33	25
	WIP	E (12)	25 (1.5)	0	75	42 (1.4)	8	50
	F	B (6)	33 (2.3)	0	67	NA	33	67
	F	M (6)	17 (1.5)	0	83	33 (1.7)	0	67
	F	E (6)	17 (1.0)	0	83	NA	0	100
Total/mean		72	18.1 (1.5)	2.8	79.1	22.2 (1.4)	13.9	63.9
D (WPC-80)	R	B (8)	25 (1.0)	0	75	13 (1.7)	13	74
	R	M (6)	33 (1.2)	17	50	NA	17	83
	R	E (6)	NA	17	83	33 (1.2)	50	17
	WIP	B (23)	22 (1.9)	52	26	9 (1.7)	61	30
	WIP	M (23)	13 (2.0)	65	22	9 (2.2)	45	46
	WIP	E (24)	8 (2.5)	67	25	17 (2.2)	42	41
	F	B (5)	NA	100	0	20 (2.6)	80	0
	F	M (5)	20 (2.3)	80	0	20 (2.3)	80	0
	F	E (6)	33 (2.5)	67	0	NA	100	0
Total/mean		106	16.0 (1.9)	54.7	29.3	12.3 (2.0)	50.0	37.7
Total average		414	59.4 (2.8)	18.1	22.5	37.8 (1.8)	33.2	29.0

¹R = raw; WIP = work in process; F = finished product.²B = beginning (sampled within 2 h of start of run); M = middle (sampled within 2 h of midpoint of run); E = end (sampled within last 2 h of run).³Refers to samples with growth on agar plates after thermal enrichment and incubation at 32°C for MSC and 55°C for TSC.⁴Sample size (n) for plant B, raw, MSC was 5.⁵Total/mean = total samples per plant; and mean percentage of samples or the mean log cfu/mL or log cfu/g.⁶NA = not applicable.

positive following enrichment (Table 3). By comparison, after SP treatment (80°C for 12 min), 59.8 and 60.0% of WIP and FP samples, respectively, were positive for thermophilic sporeforming bacteria on direct plating; an additional 22.0 and 20.0% of these WIP and FP samples were positive after enrichment (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7363>). These data indicate that thermophilic sporeformers are the key sporeformer group found in dairy powder products and that a high percentage of samples are positive for thermophilic sporeformers after either SP or HHR spore treatment (80°C for 12 min and 100°C for 30 min, respectively).

Dairy powders are used in a wide range of products including bakery items, infant products, processed meats and seafood, beverages, pet foods, canned foods, and UHT products. The presence of thermophilic spores can have a negative effect on the quality and shelf life of these finished products. Several methods are used to enumerate spores in dairy powder products. Among the 3 distinct heat treatments that are most often used to allow for subsequent enumeration of spores in dairy powders, we applied 2, including (1) 80°C for 12 min (spore treatment according to SMEDP; Frank and Yousef, 2004), and (2) 100°C for 30 min (HHR spore treatment); some researchers have suggested that HHR spores relevant to dairy powders may be isolated using a spore heat treatment of 106°C for 30 min, followed by bacterial isolation and enumeration at 55°C (i.e., the ISO/TS 27265:2009 method; ISO-IDF, 2009). This method selects for the “specially [*sic*] thermoresistant spores” (STR) of *Anoxybacillus* spp. and *Geobacillus* spp., which are known to cause defects in canned and UHT products. However, certain mesophilic *Bacillus* spp. (e.g., *B. subtilis*, *B. pumilus*, and *B. licheniformis*) can also grow at temperatures that are used to detect and enumerate thermophilic organisms (i.e., 55°C); importantly, spores of these organisms seem to be inactivated by the STR spore treatment (106°C for 30 min), whereas they survive the HHR spore treatment. Although the STR method may select for thermophilic organisms that would be of primary concern in dairy powders destined for UHT or retorted products, as described by Burgess et al. (2010), we surmised that the HHR treatment represented a more appropriate approach for identification and quantification of all spores that can grow at 55°C. Importantly, the differences between the STR and HHR spore treatments also need to be considered when comparing the TSC data reported here to TSC data that may have been generated after an initial STR treatment. The apparent lack of standardization in the selection and application of these different approaches to determine spore loads in the dairy powder industry also makes it paramount for

both manufacturers and customers to understand the available laboratory methods used to enumerate spore-forming organisms and to interpret and compare data created with different methods correctly. As the specific types of sporeformers that represent finished product quality concerns depend on the specific products manufactured, future research will be needed to define the specific target sporeformers of concern in different finished products to allow for more targeted control strategies and rational powder quality specifications.

Spore Load in Dairy Powders

The dairy powders tested in the current study included SWP, NDM, AW, and WPC-80, which have very different product characteristics and are subjected to different processing conditions. It is therefore not surprising that our results indicate that spore loads in these 4 products varied considerably. For example, after SP treatment, 94.6 and 87.7% of SWP and NDM, respectively, were positive for TSC after direct plating (Table 2). Conversely, only 18.1 and 16.0% of AW and WPC-80, respectively, were positive for TSC after direct plating (Table 2). A similar trend was identified for MSC, with SWP and NDM showing a higher percentage of samples positive by direct plating compared with AW and WPC-80 (Table 2). Thus, SWP and NDM generally showed higher spore loads than AW and WPC-80.

The HHR TSC spore data showed a similar pattern, with a much larger percentage of SWP and NDM being positive following direct plating (60.7 and 54.5%, respectively) compared with AW and WPC-80 samples (0 and 2.3% positive following direct plating; Table 3). The sporeformers for which the HHR treatment selects (i.e., *A. flavithermus* and *Geobacillus* spp.) are more likely to grow at the incubation temperature used in the TSC test (55°C) compared with those selected by the MSC test (32°C). Therefore, not surprisingly, all powder products subjected to the HHR treatment had a lower incidence of HHR MSC, ranging from a low of 0% positive after direct plating in AW to a high of 13.5% positive after direct plating in SWP (Table 3).

Spore Counts from Raw to Finished Product

As expected, our results for SWP and NDM indicated that SP and HHR spore counts increased as the material moved from raw product through the finished product continuum (Figures 2A and 2B; data for AW and WPC-80 not shown because of the low incidence of sporeformers). This increase in spore counts may be attributed to at least 2 mechanisms: (1) physical concentration of the product (liquid to powder) and (2)

Table 3. Summary of highly heat resistant (HHR) thermophilic and mesophilic spore counts in dairy powders from 4 dairies in the northeast United States sampled 6 times over a 12-mo period

Plant (product)	Status ¹	Time point ² (no. of samples)	HHR thermophilic spore count (TSC)			HHR mesophilic spore count (MSC)		
			Samples positive (%)		Samples negative (%)	Samples positive (%)		Samples negative (%)
			After direct plating (mean log cfu/mL or /g)	After enrichment ³		After direct plating (mean log cfu/mL or /g)	After enrichment	
A (sweet whey)	WIP	B (24)	54 (2.3)	8	38	17 (1.3)	38	45
	WIP	M (24)	67 (2.1)	8	25	21 (1.1)	33	46
	WIP	E (23)	61 (2.5)	9	30	4 (1.3)	39	57
	F	B (6)	33 (3.3)	50	17	17 (2.0)	33	50
	F	M (6)	67 (3.2)	17	16	NA ⁴	50	50
	F	E (6)	83 (3.1)	0	17	17 (2.5)	17	66
Total/mean ⁵		89	60.7 (2.5)	11.2	28.1	13.5 (1.4)	36.0	50.5
B (NFDMM)	WIP	B (24)	33 (1.8)	63	4	21 (2.1)	42	37
	WIP	M (24)	42 (2.3)	54	4	4 (1.3)	79	17
	WIP	E (22)	82 (2.5)	18	0	5 (1.3)	82	13
	F	B (6)	67 (2.0)	33	0	NA	83	17
	F	M (6)	33 (1.9)	67	0	NA	67	33
	F	E (6)	100 (3.1)	0	0	NA	67	33
Total/mean ⁵		88	54.5 (2.3)	43.2	2.3	8.0 (1.9)	68.2	23.8
C (acid whey)	WIP	B (12)	NA	0	100	NA	8	92
	WIP	M (12)	NA	17	83	NA	0	100
	WIP	E (12)	NA	0	100	NA	8	92
	F	B (6)	NA	0	100	NA	0	100
	F	M (6)	NA	0	100	NA	0	100
	F	E (6)	NA	0	100	NA	17	83
Total/mean ⁵		54	NA	3.7	96.3	NA	5.6	94.4
D (WPC-80)	WIP	B (23)	4 (2.6)	43	53	4 (1.0)	22	74
	WIP	M (23)	4 (2.3)	52	44	4 (2.1)	13	83
	WIP	E (24)	NA	46	54	8 (3.4)	25	67
	F	B (5)	NA	100	0	NA	20	80
	F	M (5)	NA	100	0	NA	20	80
	F	E (6)	NA	83	17	NA	17	83
Total/mean		86	2.3 (2.5)	55.8	41.9	4.7 (2.5)	19.8	75.5
Total average		317	32.8 (2.4)	30.9	36.3	7.3 (1.7)	35.3	57.4

¹WIP = work in process; F = finished product. Raw materials were not analyzed for highly heat resistant spores.

²B = beginning (sampled within 2 h of start of run); M = middle (sampled within 2 h of midpoint of run); E = end (sampled within last 2 h of run).

³Refers to samples with growth on agar plates after thermal enrichment and incubation at 32°C for MSC and 55°C for TSC.

⁴NA = not applicable.

⁵Total/mean = total samples per plant; and mean percentage of samples or the mean log cfu/mL or log cfu/g.

the introduction of additional microorganisms not present in the raw material and acquired from the processing environment or conditions therein (e.g., through biofilm slough-off or environmental contamination). The contribution of the first mechanism, the physical concentration of fluid milk or liquid whey into finished powder, is easily evaluated in theory by examining the concentration factors for these 2 products. Considering that 100 g of skim milk results in 10 g of NDM and 100 g of sweet whey results in 6 g of SWP and applying the raw to finished product concentration factor to the SWP data in our study, we would expect, for example, that the raw material with a mean TSC of 2.7 log cfu/mL (Figure 2A, based on SP treatment) would result in finished SWP product with a mean TSC of 3.9 log cfu/g (Figure 2A), if the only contributing factor to the finished product count was the physical concentration of the raw material. Based on the actual finished product mean TSC of 4.2 log cfu/g at time point B (Figure 2A), it appears that physical concentration of the product may be the primary contributing mechanism. We cannot exclude, however, contributions to TSC in the finished product from spores entering the product throughout the processing continuum (e.g., the processing plant environment). Further, at time point E, the average TSC count in the finished SWP products was 4.3 log cfu/g, with a raw material count of 2.6 log cfu/g; at this time point, the finished spore counts are thus numerically higher than what would be expected if no additional contamination from external sources occurred. Specifically, TSC counts in the finished product were 0.48 log cfu/g higher for time point E than would be expected if no additional contamination from external sources occurred (Figure 2A).

Our data for NDM, which has a concentration factor from raw to finished product of 10:1, show that for the B and M time points (Figure 2B), the finished product TSC (after SP treatment) appeared to be consistent with what would be expected if all spores in the raw material survived with no additional contamination from environmental sources. Specifically, for the B time point, the average raw skim milk TSC was 1.6 log cfu/mL, whereas the average finished product TSC was 2.6 log cfu/g; at the M time point, the raw milk TSC was 1.3 log cfu/mL and the finished product TSC was 2.3 log cfu/g (Figure 2B), consistent with a 10-fold concentration factor. However, at the E time point, we observed an increase from 1.5 log cfu/mL in the raw material to 3.3 log cfu/g in the finished product (Figure 2B). This 1.8 log cfu/g increase exceeds the 1-log increase that would be predicted, suggesting additional spore contamination from environmental sources specifically at the end time point, and indicating an increase in spore load over the course of production runs in the facility.

Figure 2. Mean thermophilic spore count (TSC, log cfu/mL or log cfu/g; performed after spore pasteurization treatment) for (A) plant A (sweet whey) and (B) plant B (nonfat dry milk) samples that were positive on direct plating, by product status: raw material, work-in-process, and finished product; and through at 3 time points: beginning (B), middle (M), and end (E) of the production run. The sample size (number of samples positive on direct plating) for each category is indicated directly below each status bar, and the number above each bar represents the mean TSC value.

Although the examples detailed above are based on calculations that do not necessarily represent the real conditions that occur during processing (e.g., because we cannot sample the specific raw material that produces a given finished product samples tested), they demonstrate the factors that may be contributing to the spore load observed in the finished product. Additional work (e.g., subtyping of the organisms isolated throughout the processing continuum) is necessary to further probe the contributions, to finished product

spore loads, of raw material spore loads and spore contamination from the processing environment, including microbial growth and biofilm development. Also, further investigation is warranted to determine potential points of entry and factors leading to biofilm formation and persistence.

Overall, our results did not show a significant increase in the incidence of MSC, TSC, HHR MSC, or HHR TSC (P -values ranging from 0.07 to 0.74) over the duration of a processing run (from beginning to end) in any of the products tested. This is despite the numerical evidence detailed above, which suggests some higher TSC in the plant producing NDM in the finished product compared with that at the beginning of production. In comparison, previous work (Scott et al., 2007) found substantial increases in spore load (>3.0 log cfu/g) throughout a processing run, with the plate heat exchanger identified as the primary location of in-plant spore contamination. Scott et al. (2007) implicated the buildup and sloughing-off of foulant or the accumulation of layers of burnt milk during a processing run, as potential sources of contamination, and they identified high levels of *Geobacillus* spp. in the foulant samples. Our data indicate that in 3 of the plants in this study (those producing SWP, AW, and WPC-80), there was little foulant slough-off or perhaps that slough-off occurred intermittently during the processing run, which may explain the lack of increase in bacterial load during the processing run. In the NDM plant, however, we found evidence that biofilm slough-off or other bacterial contamination may have contributed to an increased spore load (specifically an increased TSC) near the end of the process.

CONCLUSIONS

Thermophilic sporeformers are the primary organisms of concern in dairy powders. Thermophilic sporeformers were more prevalent than both mesophilic and psychrotolerant sporeformers in all dairy powders tested. Additionally, the incidence of HHR sporeformers was lower than that of other sporeformers tested. Spore counts of WIP and FP varied greatly by product type; however, sporeformer counts in raw materials remained reasonably constant throughout the run. The products with the highest thermophilic spore counts were NDM and SWP, indicating a specific need to target spore interventions for these products. Stringent specifications for sporeformers in dairy powders, which are being used by many customers, may be a hurdle to expanding the market for NDM and SWP. As the demand for dairy powders increases globally, understanding the presence and transmission of thermophilic sporeformers is of great importance to the dairy industry.

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REFERENCES

- Bienvenue, A. 2013. Opportunities for low-spore milk powder in a global marketplace. US Dairy Industry Spore Seminar, San Francisco, CA. Accessed Jun. 11, 2013. <http://usdec.files.cms-plus.com/PDFs/2013SporeSeminar/01-OpportunitiesforLow-Spore-MilkPowderinaGlobalMarketplace.pdf>.
- Bloomfield, S. F., and M. Arthur. 1994. Mechanisms of inactivation and resistance of spores to chemical biocides. Soc. Appl. Bacteriol. Symp. Ser. 23:91S–104S.
- Boor, K. J., D. P. Brown, S. C. Murphy, S. M. Kozlowski, and D. K. Bandler. 1998. Microbiological and chemical quality of raw milk in New York State. J. Dairy Sci. 81:1743–1748.
- Bower, C. K., J. McGuire, and M. A. Daeschel. 1996. The adhesion and detachment of bacteria and spores on food-contact surfaces. Trends Food Sci. Technol. 7:152–157.
- Burgess, S. A., J. D. Brooks, J. Rakonjac, K. M. Walker, and S. H. Flint. 2009. The formation of spores in biofilms of *Anoxybacillus flavithermus*. J. Appl. Microbiol. 107:1012–1018.
- Burgess, S. A., D. Lindsay, and S. H. Flint. 2010. Thermophilic bacilli and their importance in dairy processing. Int. J. Food Microbiol. 144:215–225.
- Christiansson, A., J. Bertilsson, and B. Svensson. 1999. *Bacillus cereus* spores in raw milk: Factors affecting the contamination of milk during the grazing period. J. Dairy Sci. 82:305–314.
- Collins, E. B. 1981. Heat-resistant psychrotrophic organisms. J. Dairy Sci. 64:157–160.
- Crielly, E. M., N. A. Logan, and A. Anderton. 1994. Studies on the *Bacillus* flora of milk and milk products. J. Appl. Bacteriol. 77:256–263.
- De Vos, P., G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K. Schleifer, and W. B. Whitman. 2009. Bergey's Manual of Systematic Bacteriology. Vol. 3. The Firmicutes. Springer, New York, NY.
- Durak, M. Z., H. I. Fromm, J. R. Huck, R. N. Zadoks, and K. J. Boor. 2006. Development of molecular typing methods for *Bacillus* spp. and *Paenibacillus* spp. isolated from fluid milk products. J. Food Sci. 71:50–56.
- Faille, C., F. Fontaine, and T. Bénézech. 2001. Potential occurrence of adhering living *Bacillus* spores in milk product processing lines. J. Appl. Microbiol. 90:892–900.
- Flint, S. H., P. J. Bremer, and J. D. Brooks. 1997. Biofilms in dairy manufacturing plants—Description, current concerns and methods of control. Biofouling 11:81–97.
- Flint, S. H., J. Palmer, K. Bloemen, J. Brooks, and R. Crawford. 2001a. The growth of *Bacillus stearothermophilus* on stainless steel. J. Appl. Microbiol. 90:151–157.
- Flint, S. H., L. J. H. Ward, and K. M. R. Walker. 2001b. Functional grouping of thermophilic *Bacillus* strains using amplification pro-

- files of the 16S–23S internal spacer region. *Syst. Appl. Microbiol.* 24:539–548.
- Frank, J. F., and A. E. Yousef. 2004. Tests for groups of microorganisms. Pages 227–248 in *Standard Methods for the Examination of Dairy Products*. 17th ed. H. M. Wehr and J. F. Frank, ed. Am. Public Health Assoc., Washington, DC.
- Franklin, J. G., D. J. Williams, and L. F. L. Clegg. 1956. A survey of the number and types of aerobic mesophilic spores in milk before and after commercial sterilization. *J. Appl. Microbiol.* 19:46–53.
- Fromm, H. I., and K. J. Boor. 2004. Characterization of pasteurized fluid milk shelf-life attributes. *J. Food Sci.* 69:207.
- Graham, T. 2004. Sampling dairy and related products. Pages 63–92 in *Standard Methods for the Examination of Dairy Products*. 17th ed. H. M. Wehr and J. F. Frank, ed. Am. Public Health Assoc., Washington, DC.
- Griffiths, M. W., and J. D. Phillips. 1990. Incidence, source and some properties of psychrotrophic *Bacillus* spp found in raw and pasteurized milk. *Int. J. Dairy Technol.* 43:62–66.
- Hood, S. K., and E. A. Zottola. 1995. Biofilms in food processing. *Food Contr.* 6:9–18.
- Huck, J. R., B. H. Hammond, S. C. Murphy, N. H. Woodcock, and K. J. Boor. 2007. Tracking spore-forming bacterial contaminants in fluid milk-processing systems. *J. Dairy Sci.* 90:4872–4883.
- Huck, J. R., M. Sonnen, and K. J. Boor. 2008. Tracking heat-resistant, cold-thriving fluid milk spoilage bacteria from farm to packaged product. *J. Dairy Sci.* 91:1218–1228.
- ISO-IDF. 2009. Dried milk: Enumeration of the specially thermoresistant spores of thermophilic bacteria. International Organization for Standardization (ISO), Geneva, Switzerland, and International Dairy Federation (IDF), Brussels, Belgium.
- Ivy, R. A., M. L. Ranieri, N. H. Martin, H. C. den Bakker, B. M. Xavier, M. Wiedmann, and K. J. Boor. 2012. Identification and characterization of psychrotolerant sporeformers associated with fluid milk production and processing. *Appl. Environ. Microbiol.* 78:1853–1864.
- Klijn, N., L. Herman, L. Langeveld, M. Vaerewijck, A. A. Wagendorp, I. Huemer, and A. H. Weerkamp. 1997. Genotypical and phenotypical characterization of *Bacillus sporothermodurans* strains, surviving UHT sterilisation. *Int. Dairy J.* 7:421–428.
- Klijn, N., F. F. Nieuwenhof, J. D. Hoolwerf, C. B. van der Waals, and A. H. Weerkamp. 1995. Identification of *Clostridium tyrobutyricum* as the causative agent of late blowing in cheese by species-specific PCR amplification. *Appl. Environ. Microbiol.* 61:2919–2924.
- Kumar, C. G., and S. K. Anand. 1998. Significance of microbial biofilms in food industry: A review. *Int. J. Food Microbiol.* 42:9–27.
- Labots, H., G. Hup, and T. E. Galesloot. 1965. *Bacillus cereus* in raw and pasteurized milk. III. The contamination of raw milk with *B. cereus* spores during its production. *Neth. Milk Dairy J.* 19:191–221.
- Magnusson, M., A. Christiansson, and B. Svensson. 2007. *Bacillus cereus* spores during housing of dairy cows: Factors affecting contamination of raw milk. *J. Dairy Sci.* 90:2745–2754.
- Marchand, S., J. De Block, V. De Jonghe, A. Coorevits, M. Heyndrickx, and L. Herman. 2012. Biofilm formation in milk production and processing environments; influence on milk quality and safety. *Comp. Rev. Food Sci. Food Safety* 11:133–147.
- Martin, N. H., M. L. Ranieri, S. C. Murphy, R. D. Ralvea, M. Wiedmann, and K. J. Boor. 2011. Results from raw milk microbiological tests do not predict the shelf-life performance of commercially pasteurized fluid milk. *J. Dairy Sci.* 94:1211–1222.
- Muir, D. D., M. W. Griffiths, and P. M. Kelly. 1999. Growth of thermophilic spore forming bacilli in milk during the manufacture of low heat powders. *Int. J. Dairy Technol.* 52:45–50.
- Palmer, J. S., S. H. Flint, J. Schmid, and J. D. Brooks. 2010. The role of surface charge and hydrophobicity in the attachment of *Anoxybacillus flavithermus* isolated from milk powder. *J. Ind. Microbiol. Biotechnol.* 37:1111–1119.
- Parkar, S. G., S. H. Flint, and J. D. Brooks. 2003. Physiology of biofilms of thermophilic bacilli—Potential consequences for cleaning. *J. Ind. Microbiol. Biotechnol.* 30:553–560.
- Parkar, S. G., S. H. Flint, J. S. Palmer, and J. D. Brooks. 2001. Factors influencing attachment of thermophilic bacilli to stainless steel. *J. Appl. Microbiol.* 90:901–908.
- Postollec, F., A. G. Mathot, M. Bernard, M. L. Divanac'h, S. Pavan, and D. Danièle. 2012. Tracking spore-forming bacteria in food: From natural biodiversity to selection by processes. *Int. J. Food Microbiol.* 158:1–8.
- Quiberoni, A., D. Guglielmotti, and J. Reinheimer. 2008. New and classical spoilage bacteria causing widespread blowing in Argentinean soft and semihard cheeses. *Int. J. Dairy Technol.* 61:358–363.
- Ralvea, R. D., M. Wiedmann, and K. J. Boor. 1998. Bacterial tracking in a dairy production system using phenotypic and ribotyping methods. *J. Food Prot.* 61:1336–1340.
- Ranieri, M. L., J. R. Huck, M. Sonnen, D. M. Barbano, and K. J. Boor. 2009. High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk. *J. Dairy Sci.* 92:4823–4832.
- Ranieri, M. L., R. A. Ivy, W. R. Mitchell, E. Call, S. N. Masiello, M. Wiedmann, and K. J. Boor. 2012. Real-time PCR detection of *Paenibacillus* spp. in raw milk to predict shelf life performance of pasteurized fluid milk products. *Appl. Environ. Microbiol.* 78:5855–5863.
- Ridgway, J. D. 1954. A note on the seasonal variations of the keeping quality of commercial sterilized milk. *J. Appl. Microbiol.* 17:1–5.
- Ridgway, J. D. 1955. Some recent observations on the bacteriology of sterilized milk. *J. Appl. Microbiol.* 18:374–387.
- Ronimus, R. S., L. E. Parker, N. Turner, S. Poudel, A. Rückert, and H. W. Morgan. 2003. A RAPD-based comparison of thermophilic bacilli from milk powders. *Int. J. Food Microbiol.* 85:45–61.
- Russell, A. D. 1990. Bacterial spores and chemical sporicidal agents. *Clin. Microbiol. Rev.* 3:99–119.
- Scheldeman, P., L. Herman, S. Foster, and M. Heyndrickx. 2006. *Bacillus sporothermodurans* and other highly heat-resistant spore formers in milk. *J. Appl. Microbiol.* 101:542–555.
- Scott, S. A., J. D. Brooks, J. Rakonjac, K. M. R. Walker, and S. H. Flint. 2007. The formation of thermophilic spores during the manufacture of whole milk powder. *Int. J. Dairy Technol.* 60:109–117.
- Seale, R. B., R. Dhakal, K. Chauhan, H. M. Craven, H. C. Deeth, C. J. Pillidge, I. B. Powell, and M. S. Turner. 2012. Genotyping of present-day and historical *Geobacillus* species isolates from milk powders by high-resolution melt analysis of multiple variable-number tandem-repeat loci. *Appl. Environ. Microbiol.* 78:7090–7097.
- Seale, R. B., S. H. Flint, A. J. McQuillan, and P. J. Bremer. 2008. Recovery of spores from thermophilic dairy bacilli and effects of their surface characteristics on attachment to different surfaces. *Appl. Environ. Microbiol.* 74:731–737.
- Slaghuys, B. A., M. C. te Giffel, R. R. Beumer, and G. André. 1997. Effect of pasturing on the incidence of *Bacillus cereus* spores in raw milk. *Int. Dairy J.* 7:201–205.
- te Giffel, M. C., A. Wagendorp, A. Herrewegh, and F. Driehuis. 2002. Bacterial spores in silage and raw milk. *Antonie van Leeuwenhoek* 81:625–630.
- Wong, H. C., M. H. Chang, and J. Y. Fan. 1988. Incidence and characterization of *Bacillus cereus* isolates contaminating dairy products. *Appl. Environ. Microbiol.* 54:699–702.
- Yuan, D. D., G. C. Liu, D. Y. Ren, D. Zhang, L. Zhao, C. P. Kan, Y. Z. Yang, W. Ma, Y. Li, and L. B. Zhang. 2012. A survey on occurrence of thermophilic bacilli in commercial milk powders in China. *Food Contr.* 25:752–757.
- Zottola, E. A., and K. C. Sasahara. 1994. Microbial biofilms in the food processing industry—Should they be a concern? *Int. J. Food Microbiol.* 23:125–148.