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# Prevalence of thermoduric bacteria and spores in nonfat dry milk powders of Midwest origin

**Kimberly P. Buehner,* Sanjeev Anand,*[1] and Gemechis D. Djira†**
*Midwest Dairy Foods Research Center, Dairy Science Department, and
†Department of Mathematics and Statistics, South Dakota State University, Brookings 57007

## ABSTRACT

Samples of nonfat dry milk powder were analyzed for the presence of heat-resistant bacteria. The samples were collected from Midwest manufacturing companies and were evaluated for the presence of spores, thermoduric bacteria, and the total bacterial count. Three companies were included in this study, and results showed differences between each of the companies in the heat-resistant microbial groups tested. Company 3 had the highest levels of total spores and thermoduric bacteria: 3.6 ± 0.14 and 3.5 ± 0.13 log cfu/g, respectively. Interestingly, this company did not have the highest total bacterial count but rather the second lowest total bacterial count for the group, perhaps because of the higher proportion of thermophiles present in the powders from this company. The average level of total bacterial counts was 2.57 ± 0.07 log cfu/g. Isolates obtained from the samples were identified by mass spectrometry, and all of the companies showed *Bacillus licheniformis* as the most prevalent bacterial species identified.
**Key words:** spore, thermoduric bacteria, highly heat-resistant spores, *Bacillus*

## INTRODUCTION

Several *Bacillus* species are commonly present in milk at all stages of processing (Crielly et al., 1994; Scheldeman et al., 2005) and, along with other thermoduric organisms, have the potential to contaminate processed dairy products. Aerobic bacterial counts in milk powders include thermophilic bacilli, such as *Bacillus*, *Anoxybacillus*, and *Geobacillus*, for which, real-time PCR assays have been developed (Rückert et al., 2006). Such bacteria with the ability to form spores (**SP**) pose a challenge to the quality of milk and milk products. Spores are essentially dormant cells that can germinate, or become activated, in very short periods and produce fully functioning viable cells (Doyle et al., 1997). In these dormant cells, the spore-coat consists of a complex protein structure that is not found in vegetative cells (Driks, 2002; Henriques and Morgan, 2007). The thickness of the spore-coat varies between species, as does the actual chemical and physical make-up of this structure. Different species of SP will have varying degrees of resistance to cleaning agents, adverse pH, and temperatures, along with other unideal environmental conditions capable of damaging vegetative cells (Doyle et al., 1997; Driks 2002). Some of the SP are capable of surviving not only pasteurization, but even UHT and spray-drying processes. As such, it becomes important to control raw milk contamination of bacteria with this ability to form SP in the raw milk, which will lead to an improvement in the quality of any dairy products processed from milk.

A previous study took samples of raw milk all the way through pasteurization, concentration, and spray drying to the final end product of skim milk powder (**SMP**). The levels of thermoduric bacteria (**TDB**), SP, and psychrotrophs were enumerated (Griffiths et al., 1988). The TDB and SP are not eliminated during powder manufacture and can even become concentrated from the initial counts already present in the raw milk. Another study followed skim milk to final powder manufacture and showed starting levels of thermophilic bacteria ranged from 350 to 3,500 cfu/mL and SP from 200 to 2,000 cfu/mL (Murphy et al., 1999). These samples, however, were ready-to-process milk rather than raw milk. Nonetheless, the samples still contained significant amounts of thermophilic bacteria and SP.

A large majority of the bacteria recovered from milk powders are classified under the *Bacillus* species. In a large study encompassing powders from 18 different countries, 92% of the bacteria recovered from the samples were classified as *Geobacillus stearothermophilus*, *Bacillus licheniformis*, and *Anoxybacillus flavithermus* (Rückert et al., 2004). *Bacillus subtilis* has also been found to be a fairly ubiquitous microorganism in global milk powders (Ronimus et al., 2003). These strains can grow at elevated temperatures and are classified as thermophilic bacteria, which allows them to prolifer-

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[1]Corresponding author: Sanjeev.anand@sdstate.edu

ate even during milk processing. Some areas such as the regeneration sections of the evaporator, preheater, and heat exchangers, among others, lie within optimum growing temperatures for thermophiles such as these (Murphy et al., 1999; Scott et al., 2007; Gleeson et al., 2013). It has also been reported that the sporeforming bacteria are able to grow during the holding period between milk powder recombining and UHT processing; however, the level of SP remain the same if the holding period is less than 8 h (Schwarzenbach and Hill, 1999). Other studies have commonly identified strains of *G. stearothermophilus*, *A. flavithermus*, and *B. subtilis*, along with *B. licheniformis*, as contaminants in milk powders from several European countries (Rückert et al., 2004).

A study conducted with samples from 4 different US powder-manufacturing companies tested the levels of sporeformers, thermophiles, and coliforms in nonfat dry milk (**NFDM**) and SMP. The study found that in low-heat NFDM and SMP, thermophilic sporeformers reached a maximum of 4.1 log cfu/g and thermophilic bacteria numbers ranged from 2.0 to 2.9 log cfu/g. All of the NFDM and SMP samples contained <10 cfu/g for coliforms, thermoresistant bacteria, and yeasts and molds (Ali et al., 2013). No set standards in the United States exist for milk powders as far as acceptable levels of thermophilic bacteria or sporeformers, but general quality parameters exist in the industry limiting thermophilic sporeformers to a maximum of 2,000 cfu/g, thermophilic bacteria to <10,000 cfu/g, and thermoresistant SP (100°C, 30 min) to <500 cfu/g (Wehr and Frank, 2004). Because no current standards exist, the variability in levels of TDB and sporeformers (thermophilic and mesophilic) present in NFDM, SMP, and other milk powders needs to be studied more in depth to establish an industry standard. Such standards are important to the dairy industry because after reconstitution of milk powders, SP are more likely to return to their vegetative state because of higher water content, which is vital for any form of vegetative growth and proliferation. The integrity of any reconstituted milk powder is a product of water quality and processing conditions, along with the original microbial content of the milk powder among other considerations.

This study was performed to identify heat-tolerant bacterial contaminants in milk powders produced in the Midwest region and to enumerate total bacteria counts (**TBC**), SP, and TDB present in dairy powders. Because the bacterial contaminants in raw milk are quite variable because of regional differences in climate, strategies used to reduce the levels of heat-tolerant bacterial contaminants in NFDM will vary depending on source of raw milk and processing conditions. This study encompasses the first step of this process, which is the identification and enumeration of potential milk-powder contaminants in the Midwest region.

## MATERIALS AND METHODS

### Source of Powder Samples

Nonfat dry milk samples were obtained from 3 powder-manufacturing companies operating in the Midwest region and were representative of a total of 39 lots (company 1, 11 lots; company 2, 18 lots; company 3, 10 lots). The samples were organized by lot numbers and stored at room temperature until analysis.

### Sample Preparation

From samples collected, subsamples of 11 ± 0.2 g samples were weighed out and dissolved into 99 mL of 2% sodium citrate (Remel, Thermo Scientific, Waltham, MA). The diluent was warmed to about 50°C before the experiment to improve the solubility of the powder samples. The samples were split into equal 30-mL portions and placed into sterile screw-cap tubes. The tubes were subjected to the following heat treatments: 63 ± 0.5°C for 30 min (TDB) and 80 ± 0.5°C for 12 min (SP). The remaining sample was plated in duplicate without heat treatment for TBC. The heat-treated samples were cooled in an ice bath for 10 min before plating (Downes and Ito, 2001).

### Microbiological Analysis

The samples were diluted using phosphate buffer solution and plated using brain heart infusion (Remel) agar using the pour plating technique. From each heat treatment the samples were directly plated in duplicate and incubated at 32°C (mesophilic counts for TDB and SP) and 55°C (thermophilic counts for TDB and SP) for 48 h before enumeration to give as much recovery time as possible to potentially allow injured cells to grow. The TBC plates were incubated only at 32°C for 48 h. Each lot was plated in triplicate with the average of these trials reported as the lot count, and for each company, lot counts were averaged to report manufacturer's counts.

### Isolate Identification

Morphologically different colonies numbering 60 total were randomly selected from the plates from each heat treatment and company. The isolates were sent to the Animal Disease Research and Diagnostic Laboratory at South Dakota State University to be identified through matrix-assisted laser desorption/ionization