J. Dairy Sci. 97:6777–6784
http://dx.doi.org/10.3168/jds.2014-8342

Prevalence of thermoduric bacteria and spores on 10 Midwest dairy farms

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ABSTRACT

Thermoduric bacteria (TDB), including sporeformers and their spores, can be present in milk and dairy products even after pasteurization. They have the potential to adversely affect the quality and shelf life of products. The objectives of this study were to identify the origin and common species of heat-resistant bacteria occurring during summer and winter on Midwest dairy farms. Bulk tank milk samples were taken from 10 dairy farms located along the South Dakota section of Interstate 29, with herd sizes ranging from 650 to 3,500 lactating dairy cows. Milk samples were profiled for the prevalence of TDB and spore counts (SC). Corn silage samples and swabs of the milking clusters were also taken at the dairies to further profile the potential sources of TDB and SC. The samples were taken 3 times during 2 seasons [winter (January–March) and summer (June–August)] to track seasonal changes in the farm bacterial flora. During winter, the average TDB counts in bulk tank milk were 2.61 log compared with 2.76 log TDB counts in the summer. The SC was 1.08 log in the winter, which was half the 2.06 log SC present in the summer season. Corn silage sampled in winter contained a 7.57 log TDB count compared with an increased 10.77 log TDB count during summer sampling. Concentrations of SC in corn silage reached an average of 6.3 log in winter compared with 11.81 log for summer. The seasonal effect was evident with an increase in summer counts across the board for TDB and SC, both in the feed and bulk tank milk samples. *Bacillus licheniformis* was the predominant species identified in 62.4% of winter (85 total) and 49.4% of summer (83 total) samples. *Bacillus subtilis* made up 9.4% of the remaining winter isolates, followed by *Bacillus sonorensis* at 8.2%. Conversely, *B. sonorensis* made up 12% of the summer isolates followed by *Bacillus pumilus* at 10.8%. *Bacillus licheniformis* is a ubiquitous microbe and was isolated from both TDB and sporeformer categories in all 3 sample types. There were larger increases in SC than TDB, indicating that summer temperatures and conditions may favor proliferation of sporeforming bacteria over that of TDB. In conclusion, samples from bulk tank milk, milking cluster swabs, and corn silage samples at each of the 10 sites indicated that *B. licheniformis* was the major contaminant species, regardless of season. In this experiment, corn silage was the major environmental source of both TDB and SC with higher concentrations in summer when compared with winter.
Key words: spore, thermoduric bacteria, corn silage, *Bacillus*

INTRODUCTION

Food safety has come a long way since the days of drinking warm raw milk, fresh from the udder. While some argue this to be the healthiest and highest quality milk, current food recalls and foodborne illness outbreaks indicate the need for pasteurization. Along with safety, consumers are becoming more readily aware of food quality and insist upon products with the highest standards for excellence. As the population continues to increase, the consumption and demand for high-quality dairy products follows suit. In the United States, the Food and Drug Administration along with the USDA have declared that thermoduric, thermophilic, psychrotrophic, and spore-forming bacteria pose the greatest spoilage threat to dairy products (Hull et al., 1992). Pasteurization is a necessary step for safe consumption of fluid milk and other dairy products (Gleeson et al., 2013), but it does not fully inactivate heat-tolerant microorganisms. Thermoduric bacteria (**TDB**) have the potential to withstand pasteurization temperatures (Rückert et al., 2004; Gleeson et al., 2013). In addition, some highly heat-resistant spores can survive UHT (Harrington, 2009) and even spray-drying processes and persist in pasteurized powders (Hammer et al., 1995). Similarly, psychrotrophic bacteria have the ability to proliferate at refrigeration temperatures (Hull et al., 1992; te Giffel et al., 1995). Contamination in raw milk can arise from a variety of sources including, but not limited to soil (Slaghuis et al., 1997; Vissers et al., 2007a), feed (Hull et al., 1992; Slaghuis et al., 1997; te Giffel et al., 2002), water (Billing and Cuthbert, 1958; Torp et al., 2001), bedding (Hull et al., 1992; Torp et al., 2001; te Giffel et al., 2002), manure (Hull et al., 1992; Van Heddeghem and Vlaemynck, 1992; Fagerlund

Received May 8, 2014.
Accepted August 6, 2014.
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et al., 2004), teats (Christiansson et al., 1999), and milking equipment (Van Heddeghem and Vlaemynck, 1992; Christiansson et al., 1999; Torp et al., 2001; te Giffel et al., 2002). Spores, specifically, *Bacillus* species, are commonly present in raw milk (Crielly et al., 1994; Scheldeman et al., 2005), and in farm bulk milk tanks have reached up to 3 log (Vissers et al., 2007b; Scheldeman et al., 2005). Spore concentrations of 3 log have been reported on teat cups and milking clusters (Scheldeman et al., 2005).

The thermoduric sporeformers and their spores can be carried over to dairy products (Lukášová et al., 2001; Hill and Smythe, 2012). They are especially significant in dried products due to the concentration effect. Milk powders are highly lucrative dairy products, because of their ease of transportation and prolonged shelf life compared with fluid milk (Tetra Pak Processing Systems, 2003). Their prolonged shelf life makes them an ideal product for worldwide exports. At the present time, however, there are no international standards set for acceptable spore concentrations in milk powders. A large majority of bacteria recovered from milk powders are classified under the genus *Bacillus*. In a large study encompassing powders from 18 different countries, 92% of bacteria isolates recovered were classified as *Geobacillus stearothermophilus*, *Bacillus licheniformis*, or *Anoxybacillus flavithermus* (Scott et al., 2007). These strains are classified as thermophilic bacteria, as they could grow at high temperatures and proliferate during the regeneration sections of the pasteurizer plate and stages of the evaporator during milk processing (Scott et al., 2007). Processing milk into powder concentrates total milk solids as well as any spores or spore-forming bacteria present in the incoming raw milk (Hill and Smythe, 2012). A recent comprehensive review includes the details on aerobic sporeformers and their implications for dairy industry (Anand and Khanal, 2013).

The incidence of spores and TDB in bulk tank milk has been extensively reported in many parts of the world, but none have been recently conducted in the Midwestern United States (Boor et al., 1998; Christiansson et al., 1999; te Giffel et al., 2002; Scheldeman et al., 2005, Magnusson et al., 2006; Coorevits et al., 2008). Magnusson et al. (2006) conducted an extensive study troubleshooting different premilking teat-cleaning procedures to decrease the number of spores in the resultant bulk tank milk. A predictive modeling system created by Vissers et al. (2007b) estimated soil contamination of teats results in 33% of bulk tank milk containing greater than 1,000 spores/mL. Another study conducted in New York State found that the mean number of aerobic spores in raw milk supply was around 49 spores/mL, and the corresponding TDB was enumerated at 129 cfu/mL (Boor et al., 1998).

The present study comprised 10 dairies ranging from 650 to 3,500 lactating cows located on the eastern part of South Dakota along Interstate 29. This experiment tracked the incidences and sources of TDB and bacterial spore contamination on the 10 local dairies and the raw milk in bulk tanks that supplied milk for the Central Federal Milk Order No. 32 of the central Midwestern United States. Winter and summer samples were tested to assess the variation between both seasons. Environmental samples comprising corn silage and milking cluster swabs were also collected at 9 of the farms to track potential cross contamination into the bulk tank milk. The identity of the dairies remained confidential, but each was identified with a specific number. Each dairy was sampled a total of 3 times both in winter (January–March) and summer (June–August). Generally, no significant differences existed in the husbandry practices between seasons in the farms sampled.

MATERIALS AND METHODS

Sample Collection

Two 30-mL milk samples were taken from the bulk tank at each of the 10 sites. Environmental samples obtained from 9 of the 10 sites consisted of Quick Swabs (3M Co., St. Paul, MN) of the milking clusters and corn silage composites taken at 3 different heights of the pile. Raw milk samples were transported to the laboratory refrigerated and kept at <6°C, and then plated and incubated within 12 h of being obtained. Environmental swabs were kept refrigerated and plated within 24 h.

Raw milk samples were split into 2 screw-cap tubes of 5-mL each. Environmental swabs were displaced into 9-mL PBS dilution tubes and then split into two. Samples of 10 ± 0.5 g of corn silage were diluted in 90 mL of PBS and then placed in a stomacher for 2 min at 260 rpm (te Giffel et al., 2002) and then split in half into screw-cap tubes.

Sample Plating

Heat treatments for thermoduric bacteria counts (**TDC**) or laboratory pasteurization counts, and spore counts (**SC**) were 63 ± 0.5°C for 30 min and 80 ± 0.5°C (mean ± SE) for 10 min, respectively. Sterile 20 × 125-mm screw cap test tubes were used for water bath incubations; the water level was in contact with the entire sample without covering the test tube lid (Wehr and Frank, 2004). Samples were cooled for 10 min in an ice water bath and then pour plated in duplicate on plate count agar (Remel Inc., Lenexa, KS). Duplicates were incubated at ideal growth temperatures for each