J. Dairy Sci. 100:1–17
https://doi.org/10.3168/jds.2016-11540

The microbiome of bulk tank milk: Characterization and associations with somatic cell count and bacterial count

M. X. Rodrigues,* S. F. Lima,† S. G. Canniatti-Brazaca,* and R. C. Bicalho†[1]
*Department of Agroindustry, Food and Nutrition, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, SP 13418-900, Brazil
†Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

ABSTRACT

Numerous studies have evaluated associations between bacterial groups and milk quality parameters. However, to our knowledge, no research has been published that has analyzed associations between the microbiome and quality parameters of bulk tank milk (BTM). Thus, the aims of this study were to identify the core microbiome of BTM and to examine associations between the microbiome and milk quality parameters. Four hundred seventy-two BTM samples from 19 different dairy farms located in New York State were analyzed by next-generation sequencing and quantitative PCR of the 16S rRNA gene to assess the milk microbiome and measure total bacterial load, respectively. Flow cytometry was used to determine bacterial and somatic cell counts. Heatmaps were constructed and simple linear regressions and response screening analysis were performed. To facilitate data analysis and interpretation of the results, we dichotomized the BTM samples into high (HSCC, >200,000) and low somatic cell count (LSCC, ≤200,000) and into high (HSPC, >3.6) and low log10 SPC (LSPC, ≤3.6). Spoilage-causing, spore-forming, and pathogenic bacteria of importance to the dairy industry were identified in the core microbiome. In addition, the taxa *Thermoanaerobacterium* and *5–7N15* were identified in the core microbiome; to our knowledge, these genera have not been previously identified in milk samples. Several bacterial genera were encountered in significantly higher relative abundances in the HSCC group when compared with the LSCC group, including *Corynebacterium*, *Streptococcus*, *Lactobacillus*, *Coxiella*, *Arthrobacter*, and *Lactococcus.* Additionally, several bacterial taxa were found in significantly higher relative abundances in the HSPC groups versus the LSPC groups: *Acinetobacter*, *Enterobacteriaceae*, *Corynebacterium*, and *Streptococcus*. In addition, *Streptococcus* was highly correlated with HSPC, and this genus was the second most abundant bacterial taxon detected in samples classified as HSCC. Bacterial diversity (Shannon index) was negatively correlated with bacterial load, suggesting that the microbiomes of high-bacterial load BTM samples are dominated by smaller groups of bacterial taxa. In conclusion, the associations described corroborated current knowledge about pathogens and spoilage bacteria in relationship to milk quality, and also indicated that other bacterial taxa should be a focus of further investigations.

Key words: milk, microbiome, quality, next-generation sequencing, *Streptococcus*

INTRODUCTION

Ensuring the safety and quality of raw milk is a challenge worldwide (Gschwendtner et al., 2016). Since the 1990s, bulk tank milk (**BTM**) has been used to diagnose current and potential problems in dairy herds related to milk quality and mastitis (Jayarao et al., 2004). In addition, the food industry and cooperatives have been using BTM analysis to identify higher quality milk for which they pay premium prices (Jayarao et al., 2004; Barbano et al., 2006) based on one or more parameters (Gillespie et al., 2012). Nightingale et al. (2008) evaluated the effect of a premium program for high-quality milk in a United States cooperative focused on BTM SCC, which is a widely used criterion for milk quality premium payments (Barbano et al., 2006). Somatic cells are naturally present in milk (Li et al., 2014); commonly, SCC has been used to indicate the prevalence of IMI in dairy herds (Gillespie et al., 2012) and overall milk quality (Li et al., 2014). Associations among milk components, milk quality parameters, and presence of bacteria have been evaluated elsewhere (Park et al., 2007; Katholm et al., 2012).

Milk quality is influenced by several bacterial counts; for example, laboratory pasteurization count and SPC

Received May 31, 2016.
Accepted December 15, 2016.
[1]Corresponding author: rcb28@cornell.edu

(Gillespie et al., 2012). A high SPC in raw milk indicates mastitis occurrence and problems in milking or general hygiene (Gillespie et al., 2012). Microbial contamination of BTM can occur through several sources and by distinct microorganisms (Elmoslemany et al., 2009). Among the microorganisms, spore-forming bacteria is a concern (Barbano et al., 2006); raw milk is a source of endospores produced by mesophilic, thermophilic, and psychrotolerant spore-forming bacteria (Miller et al., 2015). Standard laboratory analysis (based on traditional culturing) following pasteurization is a good technique to indicate the level of spores (Barbano et al., 2006). The majority of microbiological analyses of raw milk microbiota have been based on culture-dependent methods (Fricker et al., 2011); however, culture-dependent methods have several limitations when compared with culture-independent methods (e.g., viable but nonculturable cells cannot be identified by culturing; Weber et al., 2014). Consequently, the use of different culture-independent methods to describe the bacterial composition has emerged. The composition of the bacterial community of raw milk has been described using different culture-independent methods (Kuang et al., 2009; Oikonomou et al., 2014; Weber et al., 2014); however, it is believed that a knowledge gap still exists in our understanding of native bacterial communities in raw milk (Fricker et al., 2011). Moreover, to our knowledge, no research has been published that has analyzed associations between the microbiome and quality parameters of BTM. Therefore, we hypothesized that the bulk tank milk microbiome will be associated with milk quality parameters (e.g., SCC and SPC).

MATERIALS AND METHODS

BTM Samples Collection

Dairy farms associated with Cayuga Marketing LLC (central region, NY), which regularly send BTM samples to Dairy One Co-op Inc. (Ithaca, NY) for milk analyses, were invited to participate in this study. Letters of consent were sent to the dairy farmers requesting permission to collect BTM samples to perform milk microbiome analyses and to use data from milk analyses carried out by Dairy One. From 29 members contacted, 19 dairy farms agreed to participate in the research, from which 472 BTM samples were obtained during the study period, September to October 2015. Trained employees collected milk samples from bulk tanks aseptically into vials, which were stored under refrigeration during transport to Dairy One Milk Laboratory. The samples submitted to Dairy One were subjected to milk quality analysis, and aliquots aseptically collected from those samples (approximately 6 mL) were sent, under refrigeration, to our laboratory at Cornell University (Ithaca, NY). The samples were received within 48 h after collection at the farms, and farm, tank, and date were recorded on a spread sheet. Samples were stored at −20°C for downstream molecular analysis.

SCC and Total Bacteria Count

The SCC and total bacterial count measurements were carried out at Dairy One Milk Laboratory. SCC was determined by flow cytometry using a Fossomatic FC Somatic Cell Counter (Foss, Hillerød, Denmark). Briefly, a mixture of each sample and staining solution was prepared, passed through a flow cell in which somatic cells emit fluorescent light pulses, and the fluorescent light pulses were counted (cells/mL). Total bacterial count was determined using a BactoScan FC+ instrument (Foss), which is also based on flow cytometry. The results for individual bacterial count were converted to colony-forming units by BactoScan FC+ software (Foss), considering SPC as reference method (International Dairy Federation, 1991; IDF Standard 100B: 1991, the reference or anchor method applied in this technology); SPC was used to express the final results for total bacterial count.

Next-Generation Sequencing of the Bacterial 16S rRNA Gene

The DNA was extracted from all samples using a PowerFood Microbial DNA Isolation Kit (MO BIO Laboratory Inc., Carlsbad, CA) following the manufacturer's protocol. The V4 hypervariable region of the bacterial/archaeal 16S rRNA gene was amplified by PCR according to a previously described protocol and optimized for the Illumina MiSeq platform (Caporaso et al., 2012) using different 12-bp error-correcting Golay barcodes for the 16S rRNA gene PCR (Lima et al., 2015). The PCR were performed using 10 μM of each primer (515F and 806R), EconoTaq Plus Green 1× Master Mix (Lucigen, Middleton, WI), 5 to 50 ng of individual metagenomic DNA samples, and ultrapure water to bring the final reaction volume to 25 μL. Blank controls in which no DNA was added to the reaction were also performed. All reactions were set up in triplicate, and the PCR conditions for amplification included an initial denaturing step of 94°C for 3 min followed by 35 cycles of 94°C for 45 s, 50°C for 1 min, and 72°C for 90 s, and a final elongation step of 72°C for 10 min. Replicates were pooled and the amplified DNA visualized by electrophoresis using 1.2% (wt/vol) agarose gels stained with 0.5 mg/mL of ethidium bromide.